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Remarks:

The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) **Interleukin-18-receptor proteins**

(57) Disclosed are a polypeptide (including that in soluble form) as receptor for a novel cytokine, i.e., interleukin-18, a DNA encoding the polypeptide, and the uses of the polypeptide including pharmaceutical and neutralizer to interleukin-18. Pharmaceuticals with the polypeptide is useful to treat and prevent autoimmune and allergic disease because it suppresses and regulates excessive immunoreaction.

EP 0 864 585 A1

Description

Background of the Invention

1. Field of the Invention

This invention relates to a novel receptor protein which recognizes a cytokine, more particularly, to a novel polypeptide which recognizes interleukin-18 (hereinafter abbreviated as "IL-18").

2. Description of the Prior Art

IL-18 is a type of cytokine or substance which mediates signal transduction in immune system. As seen in Japanese Patent Kokai Nos.27,189/96 and 193,098/96 and Haruki Okamura et al., *Nature*, Vol.378, No.6,552, pp.88-91 (1995), IL-18 was provisionally designated as "interferon-gamma inducing factor" immediately after its discovery: This designation was changed later into "IL-18" in accordance with the proposal in Shimpei Ushio et al., *The Journal of Immunology*, Vol.156, pp.4,274-4,279 (1996). IL-18 in mature form consists of 157 amino acids and possesses properties of inducing in immunocompetent cells the production of interferon-gamma (hereinafter abbreviated as "IFN- γ ") which is known as useful biologically-active protein, as well as of inducing and enhancing the generation and cytotoxicity of killer cells. Energetic studies are now in progress to develop and realize various uses of IL-18 in pharmaceuticals such as antiviral, antimicrobial, antitumor and anti-immunopathic agents which have been in great expectation because of these properties of IL-18.

As described above, in nature, cytokines including IL-18 are produced and secreted as substances responsible for signal transduction in immune system. Therefore, excessive amounts of cytokines may disturb the equilibria in immune system when they are produced or administered in the body of mammals. The surface of usual mammalian cells may bear certain sites or "receptors" which are responsible for recognition of cytokines: Secreted cytokines transduce no signal in cells till they are bound to the receptors. In normal immune system, there would be definite equilibria between respective cytokines and their receptors. Thus, in this field, with the purpose of developing and realizing IL-18 as pharmaceuticals, in addition to the clarification of physiological activities of IL-18, an expedited establishment of mass production and characterization of IL-18 receptor (hereinafter abbreviated as "IL-18R") have been in great expectation.

Summary of the Invention

In view of the foregoing, the first object of this invention is to provide a polypeptide as IL-18R which can be easily prepared on a large scale.

The second object of this invention is to provide uses of such polypeptide as pharmaceuticals.

The third object of this invention is to provide a DNA which encodes the polypeptide.

The fourth object of this invention is to provide a process to prepare the polypeptide.

The fifth object of this invention is to provide an agent to neutralize IL-18 using the polypeptide.

The sixth object of this invention is to provide a method to neutralize IL-18 using the polypeptide.

We energetically and extensively screened various means which might attain these objects, eventually resulting in the finding that a substance which recognized IL-18 was present in L428 cell, a type of lymphoblastoid cell derived from a patient with Hodgkin's disease. We isolated and characterized this substance, revealing that its nature was proteinaceous, as well as that it well recognized and bound IL-18 even when in isolated form. It was also found that the IL-18R thus identified was efficacious in treatment and prevention of various diseases resulting from excessive immunoreaction, such as autoimmune diseases, because in mammals including human, IL-18R recognized and neutralized IL-18 which activated immune system. Further, we have energetically studied L428 cell using as probe some partial amino acid sequences of the IL-18R, resulting in obtainment of a DNA which did encode IL-18R. We confirmed that a polypeptide obtained by bringing such DNAs into expression in artificial manner well recognized IL-18 and shared some essential physiological activities with the IL-18R separated from L428 cell, as well as that it was preparable in desired amounts by recombinant DNA techniques using such DNA. Thus we accomplished this invention.

More particularly, this invention attains the first object with a polypeptide as IL-18R, which is obtainable through gene expression.

This invention attains the second object with an agent for IL-18R susceptible diseases, which contains as effective ingredient such polypeptide.

This invention attains the third object with a DNA which encodes the polypeptide.

This invention attains the forth object with a process to prepare polypeptide, comprising bringing into expression a DNA which encodes the polypeptide, and collecting the resultant polypeptide.

This invention attains the fifth object with an agent to neutralize IL-18, which contains as effective ingredient the

polypeptide.

This invention attains the sixth object with a method to neutralize IL-18, characterized by allowing the polypeptide to act on IL-18.

L428 cell, which is feasible in this invention, have been deposited in the Patent Microorganism Depository, National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, 1-3, Higashi 1 chome, Tsukuba-shi, Ibaraki-ken, 305, Japan, under the accession number of "FERM BP-5777" on and after December 24th, 1996.

Brief Explanation of the Accompanying Drawings

FIG. 1 shows that the monoclonal antibody MAb #117-10C binds to L428 cells and IL-18R while competing with IL-18.

FIG. 2 is an image of intermediate tone given on display, which shows IL-18R on gel electrophoresis visualized by the Western blotting method using the monoclonal antibody MAb #117-10C.

FIG. 3 shows the inhibitory action of the monoclonal antibody MAb #117-10C on the activity of IL-18.

FIG. 4 is the chromatogram obtained by applying to IL-18R an immunoaffinity chromatography using the monoclonal antibody MAb #117-10C.

FIG. 5 is the peptide map of IL-18R.

FIG. 6 shows the structure of the recombinant DNA "pcDNA/HuIL-18R" of this invention.

FIG. 7 shows the structure of the recombinant DNA "pEFHIL18R-14" of this invention.

FIG. 8 shows the structure of the recombinant DNA "pEFHIL18RD1-2-H" of this invention.

FIG. 9 shows the structure of the recombinant DNA "pEFHIL18RD1-H" of this invention.

FIG. 10 shows the structure of the recombinant DNA "pEFMIL18RSHT" of this invention.

Throughout the Figures, the symbol "Pcmv" indicates the cytomegalo virus promoter; "EF1 α P", the elongation factor promoter; "IL-18R cDNA", the cDNA encoding the polypeptide of this invention; "EFHIL18R-14 cDNA", the cDNA encoding the soluble polypeptide of human origin according to this invention; "HIL18RD1-2-H cDNA", the cDNA encoding the soluble polypeptide of human origin according to this invention; "HIL18RD1-H cDNA", the cDNA encoding the soluble polypeptide of human origin according to this invention; and "EFMIL18RSHT cDNA", the cDNA encoding the soluble polypeptide of mouse origin according to this invention.

Detailed Description of the Invention

This invention relates to a polypeptide as IL-18R, which is obtainable through gene expression. The polypeptide of human origin according to this invention usually contains as partial amino acid sequence(s) one or more amino acid sequences of SEQ ID NOs:12 to 19: As a whole, it contains a part or whole of the amino acid sequence of SEQ ID NO: 20. While the polypeptide of mouse origin according to this invention usually contains a part or whole of the amino acid sequence of SEQ ID NO:21. Thus, the wording "polypeptide" as referred to in this invention shall include, in addition to those which wholly contain the amino acid sequence of either SEQ ID NO:20 or 21, for example, those which contain the same amino acid sequence but with addition of one or more amino acids, in particular, those which contain one or more amino acids linked to the C-and/or N-termini in SEQ ID NO:20 or 21; those which contain the same amino acid sequence as in SEQ ID NOs:20 and 21 but with deletion of one or more amino acids, in particular, soluble polypeptides which contain the amino acid sequences of SEQ ID NOs:22 to 25; and those which contain either of the amino acid sequences as described above but with a saccharide chain, as far as they are obtainable through gene expression and possess the essential functions of IL-18R. As to IL-18, those of human and mouse origins commonly consisting of 157 amino acids have been documented: Human IL-18 bears the amino acid sequence of SEQ ID NO:26 (where the amino acid with symbol "Xaa" represents either isoleucine or threonine), while mouse counterpart, the amino acid sequence of SEQ ID NO:27 (where the amino acid with symbol "Xaa" represents either methionine or threonine).

The polypeptide of this invention is usually prepared by applying recombinant DNA techniques, more particularly, by bringing into expression in artificial manner a DNA which encodes the polypeptide, and collecting the resultant polypeptide. This invention provides, in addition to a DNA which encodes the polypeptide, a process to prepare the polypeptide using recombinant DNA techniques: By practicing such a process according to this invention, desired amounts the polypeptide can be easily obtained.

The DNA which is used in this invention are those which originating natural sources, those which can be obtained by artificially modifying them and those which can be obtained through chemical synthesis, provided that they do encode the polypeptide. Generally, in this field, in case of artificially expressing DNAs which encode polypeptides, one may replace one or more nucleotides in the DNAs with different nucleotides and/or link an appropriate nucleotide sequence to the DNAs, with purpose of improving their expression efficiency and/or the physiological and physico-chemical properties of the polypeptides. Such modifications are feasible in the DNA of this invention of course: For

example, one can link to the 5'-and 3'-termini of the DNA as described above recognition sites for appropriate restriction enzymes, initiation and termination codons, promoters and/or enhancers, as far as the final polypeptide products do retain desired physiological activities. Thus, the wording "DNA" as referred to in this invention shall mean, in addition to those which encode any polypeptides as described above, those which are complementary thereto, and further those where one or more nucleotides have been replaced with different nucleotides while conserving the amino acid sequence.

To obtain such a DNA from natural sources, for example, mammalian cells including epithelial cells, endothelial cells, interstitial cells, chondrocytes, monocytes, granulocytes, lymphocytes neurocytes and their established cell lined of human and mouse origins are screened with oligonucleotides as probe which can be prepared with reference to the amino acid sequences of SEQ ID NOs:12 to 25. Examples of preferred cells are cell lines which are obtained by establishing hemopoietic cells including lymphocytes, in particular, JM cells, HDLM-2 cells, MOLT-16 cells and PEER cells described in Jun Minowada, *Cancer Review*, Vol.10, pp.1-18 (1988), and lymphoblastoid cells such as L428 cell (FERM BP-5777), KG-1 cell (ATCC CCL-246) and U-937 cells (ATCC CRL-1593.2). The human and mouse DNAs obtained in this way usually contain a part or whole of respective nucleotide sequences of SEQ ID NOs:1 and 2. For example, as shown in SEQ ID NO:7, the DNA obtained from L428 cell, a type of lymphoblastoid cell derived from a patient with Hodgkin's disease, consists of the nucleotide sequence of SEQ ID NO:1 encoding the amino acid sequence of SEQ ID NO:20, and another nucleotide sequence encoding signal peptide which is linked to the 5'-terminal in the nucleotide sequence of the SEQ ID NO:1. Soluble polypeptides with the amino acid sequences of SEQ ID NOs:22 to 25 are usually encoded by respective nucleotide sequences of SEQ ID NOs:3 to 6, which are usually used in a form where, as shown in the nucleotide sequences of SEQ ID NOs:8 to 11, a nucleotide sequence encoding signal peptide is linked to the 5'-terminal in the nucleotide sequences of SEQ ID NOs:3 to 6. Such a DNA can be also obtained through usual chemical synthesis, and in any case, DNAs can be amplified to desired levels by PCR method once they become available. By the way, the amino acid sequences of SEQ ID NOs:20 and 21 are described along with the amino acid sequences for signal peptides in P. Parnet et al., *The Journal of Biological Chemistry*, Vol.271, pp.3,967-3,970 (1996): This paper however makes neither suggestion nor teaching that the polypeptides with the amino acid sequences of SEQ ID NOs:20 and 21 do function as IL-18R.

Such DNA expresses the polypeptide when introduced into an appropriate host of microbe, animal or plant origin. The DNA of this invention is usually prepared into a recombinant DNA prior to introduction into host. Such recombinant DNA, which consists of the DNA of this invention and an autonomously replicable vector, can be easily prepared with usual recombinant DNA techniques, provided that the DNA is available. Examples of vectors which can receive the DNA of this invention are plasmid vectors including pKK223-3, pCDNA1/Amp, BCMGSNeo, pcDL-SR α , pKY4, pCDM8, pCEV4, pME18S and pEF-BOS. Autonomously replicable vectors usually comprises other nucleotide sequences, for example, promoter, enhancer, replication origin, terminator of transcription, splicing sequence and/or selection marker which facilitate the expression of the DNA of this invention in particular hosts. Expression of the DNA becomes artificially regulatable upon external stimuli when it is used in combination with either heat shock protein promoter or interferon- α promoter as disclosed in Japanese Patent Kokai No. 163,368/95 by the same applicant.

Conventional methods are feasible in the insertion of the DNA of this invention into such vector. More particularly, a gene with the DNA of this invention and an autonomously replicable vector are first digested with restriction enzyme and/or ultrasonication, then the resultant DNA and vector fragments are ligated. Ligation of DNA and vector fragments become much easier when genes and vectors are digested with restriction enzymes specific to particular nucleotides, for example, *AccI*, *BamHI*, *BstXI*, *EcoRI*, *HindIII*, *NotI*, *PstI*, *SacI*, *Sall*, *SmaI*, *SpeI*, *XbaI* and *XhoI*. To ligate DNA and vector fragments, they are first annealed, if necessary, then exposed to DNA ligase *in vivo* or *in vitro*. The recombinant DNA thus obtained is unlimitedly replicable in hosts of microbe and animal origins.

Such recombinant DNA is introduced into an appropriate host, prior to use in preparation of the polypeptide. Although conventional hosts of microbe, animal and plant origins are feasible in this invention, it is preferable to choose a host of yeast or mammalian origin in case that the final use of the polypeptide is pharmaceuticals. Examples of host cells of mammalian origin are epithelial cell, interstitial cell and hemopoietic cell of human, monkey, mouse and hamster origins, in particular, 3T3 cell (ATCC CCL-92), C1271 cell (ATCC CRL-1616), CHO-K1 cell (ATCC CCL-61), CV-1 cell (ATCC CCL-70), COS-1 cell (ATCC CRL-1650), HeLa cell (ATCC CCL-2), MOP-8 cell (ATCC CRL-1709) and their mutant strains. To introduce the DNA of this invention into such a host, one can employ conventional methods, for example, DEAE-dextran method, calcium phosphate transfection method, electroporation method, lipofection method, micro-injection method and viral infection method using retrovirus, adenovirus, herpesvirus and vaccinia virus. To select among the resultant transformants a clone which is capable of producing the polypeptide, the transformants are cultivated on culture medium, followed by selecting one or more clones where production of the polypeptide is observed. Recombinant DNA techniques using host cells of mammalian origin are detailed, for example, *Jikken-Igaku-Bessatsu, Saibo-Kogaku Handbook* (The handbook for the cell engineering), edited by Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA, published by Yodosha. Co., Ltd., Tokyo, Japan (1992), and *Jikken-Igaku-Bessatsu, Biomanual Series 3, Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), edited by Takashi YOKOTA and

Kenichi ARAI, published by Yodosha Co., Ltd., Tokyo, Japan (1993).

The transformant thus obtained produces and secretes the polypeptide inside and/or outside the host cell when cultivated on culture medium. Such cultivation is feasible with conventional culture media directed to cultivation of transformants, which are usually composed by adding to a bufferized water as base inorganic ions such as sodium ion, potassium ion, calcium ion, phosphoric ion and chloric ion; minor elements, carbon sources, nitrogen sources, amino acids and vitamins which meet to the metabolism of particular hosts; and, if necessary, sera, hormones, cell growth factors and cell adhesion factors. Particular media are, for example, 199 medium, DMEM medium, Ham's F12 medium, IMDM medium, MCDB 104 medium, MCDB 153 medium, MEM medium, RD medium, RITC 80-7 medium, RPMI-1630 medium, RPMI-1640 medium and WJJC 404 medium. One can obtain a culture product containing the polypeptide by inoculating on such a culture medium a transformant in an amount of 1×10^4 - 1×10^7 cells/ml, preferably, 1×10^5 - 1×10^6 cells/ml, and subjecting the transformant to suspension or monolayer culture at around 37°C for 1 day to 1 week, preferably, 2 to 4 days while replacing the culture medium with a fresh preparation, if necessary. The culture product thus obtained usually contains about 1 µg/l to 1 mg/l polypeptide, dependently of the type of transformant and cultivation conditions.

The culture product obtained in this way is first subjected to ultrasonication, cell-lytic enzyme and/or detergent to disrupt cells, if necessary, then polypeptides are separated from the cells or cell debris by filtration and centrifugation, followed by purification. In the purification, a culture product which has been separated from cell or cell debris is subjected to conventional methods common in purification of biologically-active proteins, for example, salting-out, dialysis, filtration, concentration, fractional precipitation, ion-exchange chromatography, gel filtration chromatography, adsorption chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis and isoelectric focusing gel electrophoresis which are used in combination, if necessary. The purified polypeptide is then concentrated and lyophilized into liquid or solid to meet to its final use. The IL-18 and monoclonal antibody, disclosed in Japanese Patent Kokai No.193,098/96 and Japanese Patent Application No.356,426/96 by the same applicant, are very useful in purification of the polypeptide: Immunoaffinity chromatographies using these do yield a high-purity preparation of the polypeptide with minimized costs and labors.

The polypeptide of this invention exhibits a remarkable efficacy in treatment and prevention of various diseases resulting from excessive immunoreaction because in mammals including human, the polypeptide recognizes and binds IL-18 which may activate immune system. Immune system, which is in nature to defend living bodies from harmful foreign substances, may cause unfavorable results in living bodies because of its nature. When mammals receive a graft of organ, for example, skin, kidney, liver, heart and bone marrow, the rejection reaction and immunoreaction against alloantigen may activate T-cells, resulting in the occurrence of inflammation and proliferation of lymphocytes. Similar phenomena are observed in case that host receives the invasion by heteroantigens, for example, allergens, which are not recognized as self. In autoimmune diseases, allergic reactions are induced by substances which must be recognized as self. The polypeptide of this invention exhibits a remarkable efficacy in treatment and prevention of various diseases resulting from such an immunoreaction because the polypeptide suppresses or regulates the immunoreaction when administered in mammals including human. Thus, the wording "susceptive diseases" as referred to in this invention shall mean all the diseases resulting from augmented immunoreaction which can be treated and/or prevented by the direct or indirect action of IL-18R: Particular susceptible diseases are, for example, rejection reactions associated with a graft of organ as described above, autoimmune and allergic diseases including pernicious anemia, atrophic gastritis, insulin-resistant diabetes, Wegener granulomatosis, discoid lupus erythematosus, ulcerative colitis, cold agglutinin-relating diseases, Goodpasture's syndrome, primary biliary cirrhosis, sympathetic ophthalmitis, hyperthyroidism, juvenile onset type diabetes, Sjögren syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, myasthenia gravis, systemic scleroderma, systemic lupus erythematosus, polyleptic cold hemoglobinuria, polymyositis, periarteritis nodosa, multiple sclerosis, Addison's disease, purpura hemorrhagica, Basedow's disease, leukopenia, Behçet's disease, climacterium praecox, rheumatoid arthritis, rheumatopyra, chronic thyroiditis, Hodgkin's disease, HIV-infections, asthma, atopic dermatitis, allergic nasitis, pollinosis and apitoxin-allergy. In addition, the polypeptide of this invention is efficacious in treatment and prevention of septic shock which results from production or administration of excessive IFN-γ.

Thus, the agent for susceptible disease, which contains as effective ingredient the polypeptides of this invention, would find a variety of uses as anti-autoimmune-diseases, anti-allergies, anti-inflammatories, immunosuppressants, hematopoietics, leukopoietics, thrombopoietics, analgesics and antipyretics directed to treatment and/or prevention of susceptible diseases as illustrated in the above. The agent according to this invention is usually prepared into liquid, suspension, paste and solid forms which contain the polypeptide in an amount of 0.00001-100 w/w %, preferably, 0.0001-20 w/w %, dependently on the forms of agents as well as on the types and symptoms of susceptible disease.

The agent for susceptible diseases according to this invention includes those which are solely composed of the polypeptide, as well as including those in composition with, for example, one or more physiologically-acceptable carriers, excipients, diluents, adjuvants, stabilizers and, if necessary, other biologically-active substances: Examples of such stabilizer are proteins such as serum albumins and gelatin; saccharides such as glucose, sucrose, lactose, maltose,

trehalose, sorbitol, maltitol, mannitol and lactitol; and buffers which are mainly composed of phosphate or succinate. Examples of the biologically-active substances usable in combination are FK506, glucocorticoid, cyclophosphamide, nitrogen mustard, triethylenethiophosphoramide, busulfan, pheniramine mustard, chlorambucil, azathioprine, 6-mercaptopurine, 6-thioguanine, 6-azaguanine, 8-azaguanine, 5-fluorouracil, cytarabine, methotrexate, aminopterin, mitomycin C, daunorubicin hydrochloride, actinomycin D, chromomycin A₃, bleomycin hydrochloride, doxorubicin hydrochloride, cyclosporin A, L-asparaginase, vincristine, vinblastine, hydroxyurea, procarbazine hydrochloride, adrenocortical hormone and auri colloid; receptor antagonists to cytokines other than IL-18, for example, antibodies respectively against interleukin-1 receptor protein, interleukin-2 receptor protein, interleukin-5 receptor protein, interleukin-6 receptor protein, interleukin-8 receptor protein and interleukin-12 receptor protein; and antagonists respectively against TNF- α receptor, TNF- β receptor, interleukin-1 receptor, interleukin-5 receptor and interleukin-8 receptor.

The agent for susceptible diseases according to this invention includes pharmaceuticals in minimal dose unit: The wording "pharmaceutical in minimal dose unit" represents those which are prepared into a physically united form suitable for prescription and also allowed to contain the polypeptide in an amount corresponding to its single dose or multiple (up to 4-fold) or divisor (up to 1/40) thereof: Examples of such form are injection, liquid, powder, granule, tablet, capsule, sublingual, ophthalmic solution, nasal drop and suppository. The agent for susceptible diseases according to this invention can be administrated through both oral and parenteral routes to exhibit in each case a remarkable efficacy in treatment and prevention of susceptible diseases. More particularly, the polypeptide is administered through oral route or parenteral route such as intradermal, subcutaneous, intramuscular or intravenous route at a dose of about 1 μ g/time/adult to about 1g/time/adult, preferably, about 10 μ g/time/adult to about 100 mg/time/adult 1 to 4 times/day or 1 to 5 times/week over 1 day to 1 year.

The DNA which encodes the polypeptide of this invention is useful in "gene therapies". Particularly, in usual gene therapies, the DNA of this invention is first inserted in a vector derived from virus such as retrovirus, adenovirus or adeno-associated virus and, alternatively, embedded in either cationic- or membrane fusible-liposomes, then the inserted or embedded DNA is directly injected in a patient with an IL-18 susceptible disease and, alternatively, introduced into lymphocytes, which have been collected from the patient, and implanted in the patient. In adoptive immunotherapy, by introducing the DNA of this invention into effector cells similarly as in the usual gene therapies, the cytotoxicity of effector cells against tumors and virus-infected cells is enhanced and this would strengthen adoptive immunotherapy. In tumor vaccine gene therapy, tumor cells, which have been extracted from a patient, are introduced with the DNA of this invention similarly as in the usual gene therapies, allowed to proliferate *in vitro* to a prescribed level and then self-transplanted to the patient: The transplanted tumor cells act as vaccine in the body of the patient, exhibiting a strong and antigen-specific antitumor immunity. Thus, the DNA of this invention exhibits a remarkable efficacy in gene therapies for various diseases including, for example, malignant tumors, viral diseases, infections and autoimmune diseases, as well as in suppression of rejection reaction and excessive immunoreaction associated with grafts of organs and allergic diseases. General procedures for gene therapies are detailed in *Jikken-Igaku-Bessatsu, Biomanual UP Series, Idenshichiryō-no-Kisogijutsu* (Basic techniques for the gene therapy), edited by Takashi SHIMADA, Izumi SAITO, and Keiya OZAWA, published by Yodosha Co., Ltd., Tokyo, Japan (1996).

Further, the polypeptide of this invention is useful in affinity chromatography and labelled assay directed to purification and detection of IL-18 because the polypeptide bears properties of recognizing and binding IL-18. In addition, the polypeptide of this invention, in particular, that in soluble form is useful in screening *in vivo* or *in vitro* agonists and antagonists to IL-18. Furthermore, the agent to neutralize IL-18 containing as effective ingredient the polypeptide and the method to neutralize IL-18 where IL-18 is exposed to the polypeptide are useful in treatment of various diseases which result from production and administration of excessive IL-18.

The following Examples are to illustrate the way of practicing this invention. The techniques employed in Examples 1 to 3 are common in this field as detailed, for example, *Jikken-Igaku-Bessatsu, Saibo-Kogaku Handbook* (The handbook for the cell engineering), edited by Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA, published by Yodosha Co., Ltd., Tokyo, Japan (1992), and *Jikken-Igaku-Bessatsu, Biomanual Series 3, Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), edited by Takashi YOKOTA and Kenichi ARAI, published by Yodosha Co., Ltd., Tokyo, Japan (1993).

Example 1

Preparation and characterization of IL-18R

Example 1-1

Preparation of IL-18R

Newborn hamsters were intraperitoneally injected with an anti-lymphocyte antibody of rabbit origin to suppress

their possible immunoreaction, subcutaneously injected at their dorsal areas with about 5×10^5 cell/animal of L428 cells (FERM BP-5777), a type of lymphoblastoid cell derived from a patient with Hodgkin's disease, and fed in usual manner for 3 weeks. The tumor masses, subcutaneously occurred, about 10g each, were extracted, disaggregated and washed in usual manner in serum-free RPMI-1640 medium (pH 7.4), thus obtaining proliferated cells.

The proliferated cells were added with a mixture solution (volume ratio of 9:1) of 0.83 w/v % NH_4Cl and 170mM Tris-HCl buffer (pH 7.7) in an amount 10-fold larger than the wet weight of the cells, stirred and collected by centrifugation at 2,000rpm for 10 minutes. The cells were then suspended in an appropriate amount of phosphate buffered saline (hereinafter abbreviated as "PBS"), stirred, collected by centrifugation at 2,000rpm, resuspended to give a cell density of about 1×10^8 cells/ml in 10mM Tris-HCl buffer (pH 7.2) with 1mM MgCl_2 and disrupted with "POLYTRON", a cell disrupter commercialized by Kinematica AG, Littau/Lucerne, Switzerland. The resultant was added with 10mM Tris-HCl buffer (pH 7.2) containing both 1mM MgCl_2 and 1M sucrose to give a final sucrose concentration of 0.2M, and centrifuged at 1,000rpm to collect the supernatant which was then centrifuged at 25,000rpm for 60 minutes, followed by collecting the precipitate. The precipitate was added with adequate amounts of 12mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (hereinafter abbreviated as "CHAPS"), 10mM ethylenediaminetetraacetic acid (hereinafter abbreviated as "EDTA") and 1mM phenylmethylsulfonylfluoride, stirred at 4°C for 16 hours, and centrifuged at 25,000rpm for 60 min, followed by collecting the supernatant.

The supernatant was charged to a column of "WHEAT GERM LECTIN SEPHAROSE 6B", a gel product for affinity chromatography commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, pre-equilibrated in PBS with 12mM CHAPS, and the column was washed with PBS containing 12mM CHAPS, and then charged with PBS containing both 0.5 M N-acetyl-D-glucosamine and 12mM CHAPS while monitoring the protein content in the eluate with the absorbance of ultraviolet at a wave length of 280nm. The fractions with an absorbance of 0.16-0.20 were collected and pooled, thus obtaining about 25 liters of aqueous solution with a protein content of about 1 mg/ml per 10^{12} starting cells.

A small portion of the solution was sampled, added with 4ng human IL-18 which had been ^{125}I -labelled in usual manner, incubated at 4°C for 1 hour, added with appropriate amounts of "POLYETHYLENE GLYCOL 6000", a polyethylene glycol preparation with an averaged molecular weight of 6,000 daltons, commercialized by E. Merck, Postfach, Germany, and allowed to stand under ice-chilling conditions for 30 minutes to effect binding reaction. The reaction product was centrifuged at 6,000rpm for 5 minutes and the resultant precipitate was collected to determine the level of radioactivity. In parallel, there was provided another sections as control in which 3μg non-labelled human IL-18 was used along with ^{125}I -labelled human IL-18 and treated similarly as above. Comparison with control revealed that the radioactivity of the precipitate from the sample solution was significantly higher. This indicated that the aqueous solution obtained in the above did contain IL-18R and the IL-18R recognized and bound IL-18 when exposed to IL-18.

Example 1-2

Binding ability to monoclonal antibody

L428 cells (FERM BP-5777) were suspended in RPMI-1640 medium (pH7.4), supplemented with 0.1 v/v % bovine serum albumin and also containing 0.1 v/v % NaN_3 , to give a cell density of 4×10^7 cells/ml, while monoclonal antibody MAb#117-10C specific to human IL-18R, obtained by the method described in Japanese Patent Application No.356,426/96 by the same applicant, was dissolved in another preparation of RPMI-1640 medium supplemented with 0.1 w/v% bovine serum albumin to give different concentrations of 0.019 μg/ml, 0.209 μg/ml, 2.3 μg/ml, 25.3 μg/ml and 139.5 μg/ml.

Fifty microliter aliquots of the cell suspension prepared in the above were mixed with 50μl of either solution with different monoclonal antibody concentrations, agitated at 4°C for 2 hours, added with 50μl of RPMI-1640 medium supplemented with 0.1 v/v % bovine serum albumin and also containing 4ng ^{125}I -labelled human IL-18 prepared in usual manner, and agitated at the same temperature for an additional 30 minutes. Subsequently, each cell suspension was added with 200μl mixture solution (volume ratio 1:1) of dibutylphthalate and dioctylphthalate and centrifuged at 10,000rpm and 20°C for 5 minutes, followed by collecting the resultant precipitates containing the cells which were then determined for radioactivity using "MODEL ARC-300", a gamma-ray counter commercialized by Aloka Co., Ltd, Tokyo, Japan.

In parallel, there were provided additional two sections where the monoclonal antibody was neglected, while 4ng ^{125}I -labelled human IL-18 was treated similarly as in the sample testing section with or without 4 micrograms of non-labelled human IL-18 (hereinafter referred to as "non-specific binding section" and "whole binding section" respectively). The levels of radioactivity found in "non-specific binding section" and "whole binding section" were put in Formula 1 together with that found in the sample testing section to calculate percent inhibition. The results were as shown in FIG. 1.

$$\text{Percent Inhibition} = \frac{(\text{Whole binding}) - (\text{Testing})}{(\text{Whole binding}) - (\text{Non-specific binding})} \times 100$$

Formula 1

Fifty microliter aliquots of an IL-18R in aqueous solution obtained by the method in Example 1-1 were added with 50 μ l solution with different concentrations for monoclonal antibody MAb #117-10C prepared similarly as above, agitated at 4°C for 2 hours, added with 4ng ¹²⁵I-labelled human IL-18, and agitated at 4°C for an additional 30 minutes. Subsequently, each mixture was added with 50 μ l of 4 mg/ml γ -globulin, allowed to stand under ice-chilling conditions for 30 minutes, added with 250 μ l of PBS with 20 w/v % polyethylene glycol, allowed to stand under ice-chilling conditions for an additional 30 minutes, and centrifuged at 6,000rpm at 4°C for 5 minutes, followed by collecting the resultant precipitates which were then determined for radioactivity similarly as above.

At the same time, there were provided additional two sections where the monoclonal antibody was neglected, while 4ng of ¹²⁵I-labelled human IL-18 were treated similarly as in the sample testing section with or without 4 μ g of non-labelled human IL-18 (hereinafter referred to as "whole binding section" and "non-specific binding section" respectively). The levels of radioactivity found in these two section were put in Formula 1 together in that found in the sample testing section to calculate percent inhibition. The results were as shown in FIG. 1.

As seen in FIG. 1, in both cases of using L428 cell and IL-18R in solution, the binding of IL-18 to L428 cell and IL-18R were inhibited much more as the concentration of monoclonal antibody MAb #117-10C elevated. This indicated that the monoclonal antibody MAb #117-10C was bound to the possible IL-18R on the surface of L428 cell in a fashion competing with IL-18, as well as that the aqueous solution obtained by the method in Example 1 did contain a protein capable of recognizing IL-18 or IL-18R and the monoclonal antibody MAb #117-10C specifically reacted with the IL-18R.

Example 1-3

Western blotting

A portion of the IL-18R in aqueous solution obtained by the method in Example 1 was sampled, added with 2/3 volume of a mixture solution of 2.5 w/v % sodium dodecyl sulfate and 50 v/v % glycerol, incubated at 37°C for 1 hour, and separated into respective proteinaceous components on conventional SDS-PAGE using 10-20% gradient gel but using no reducing agent. The proteinaceous components on the gel were transferred in usual manner to a nitrocellulose membrane which was then soaked for 1 hour in an appropriate amount of 50mM Tris-HCl buffer (pH7.5) with 10 μ g/ml of monoclonal antibody MAb #117-10C obtained by the methods described in Japanese Patent Application No.356,426/96 by the same applicant, 10 v/v % "BLOCK ACE", an immobilizing agent commercialized by Dainippon Seiyaku Co., Ltd., Osaka, Japan, and 0.05 v/v % "TWEEN 20", a detergent commercialized by City Chemical Corp., New York, U.S.A., and washed in 50mM Tris-HCl buffer (pH7.5) with 0.05 v/v % Tween 20 to remove the remaining antibody. The membrane was then soaked in Tris-HCl buffer (pH 7.5) with an appropriate amount of an anti-mouse immunoglobulin antibody of rabbit origin prelabelled with horse radish peroxidase, 10 v/v % "BLOCK ACE" and 0.05 v/v % "TWEEN 20" for 1 hour to effect reaction, washed in 50mM Tris-HCl buffer (pH 7.5) with 0.05 v/v % "TWEEN 20" and developed using "ECL kit", a kit for development commercialized by Amersham Corp., Arlington Heights, U.S.A.

At the same time, there was provided another section without the monoclonal antibody MAb #117-10C as control and it was treated similarly as above. The molecular weight markers were bovine serum albumin (67,000 daltons), ovalbumin (45,000 daltons), carbonic anhydrase (30,000 daltons), trypsin inhibitor (20,100 daltons) and α -lactalbumin (14,000 daltons). The results were as shown in FIG. 2.

In the gel electrophoresis in FIG. 2, Lane 2 (with monoclonal antibody) bore a distinct band of IL-18R which was never found in Lane 3 (without monoclonal antibody).

Example 1-4

Inhibition of IL-18 activity

KG-1 cells (ATCC CCL246), an established cell line derived from a patient with acute myelogenous leukemia, were suspended in RPMI-1640 medium (pH 7.2), supplemented with 10 v/v % fetal bovine serum and also containing 100 μ g/ml kanamycin and 18.8mM Na₂HPO₄, to give a cell density of 1 \times 10⁷ cells/ml, added with monoclonal antibody MAb #117-10C, obtained by the method described in Japanese Patent Application No.356,426/96 by the same applicant, to give a concentration of 10 μ g/ml and incubated at 37°C for 30 minutes.

The KG-1 cells in suspension were distributed on 96-well microplate to give respective amounts of 50 μ l/well, added with 50 μ l of human IL-18 which had been dissolved in a fresh preparation of the same medium to give respective con-

centrations of 0ng/ml, 1.56ng/ml, 3.12ng/ml, 6.25ng/ml, 12.5ng/ml and 25ng/ml, further added with 50μl/well of 5μg/ml lipopolysaccharide in a fresh preparation of the above medium, and incubated at 37°C for 24 hours, after which each supernatant was collected and determined for IFN-γ content by conventional enzyme immunoassay. In parallel, there were provided additional sections without the monoclonal antibody MAb #117-10C for respective IL-18 concentrations as control and they were treated similarly as above. The results were as shown in FIG. 3. The IFN-γ contents in FIG. 3 were calibrated with reference to the standardized IFN-γ preparation Gg23-901-530 available from the International Institute of Health, USA, and expressed in the International Unit(IU).

The results in FIG. 3 indicated that the presence of monoclonal antibody MAb #117-10C inhibited the induction of IFN-γ by IL-18 in KG-1 cell as immunocompetent cell. This also indicated that monoclonal antibody MAb #117-10C blocked the IL-18R on the surface of KG-1 cell in a fashion competing with IL-18, thus preventing the signal transduction of IL-18 to KG-1 cell.

Example 1-5

Purification of IL-18R

Seventy-eight milligrams of a monoclonal antibody MAb #117-10C, obtained by the method described in Japanese Patent Application No.356,426/96 by the same applicant, was dissolved in an appropriate amount of distilled water and the solution was dialyzed against borate buffer (pH 8.5) with 0.5M NaCl at 4°C for 16 hours. Thereafter, in usual manner, an appropriate amount of "CNBr-ACTIVATED SEPHAROSE 4B", a CNBr-activated gel, commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, was added to the dialyzed solution and allowed to react at 4°C for 18 hours under gentle stirring conditions to immobilize the monoclonal antibody MAb #117-10C on the gel.

The gel was packed into column in a plastic cylinder, equilibrated with 2mM CHAPS, charged with an IL-18R in aqueous solution obtained by the method in Example 1-1, and applied with PBS with 12mM CHAPS to remove non-adsorbed components. The column was then applied with 35mM ethylamine containing 2mM CHAPS (pH 10.8) while collecting the eluate in every 8ml fractions which were then checked for presence of IL-18R by the method in Example 1-1 using ¹²⁵I-labelled human IL-18. The chromatogram obtained in this operation was as shown in FIG.4.

As seen in FIG. 4, IL-18R was eluted in a single sharp peak when immunoaffinity chromatography using monoclonal antibody MAb #117-10C was applied to a mixture of IL-18R and contaminants such as the aqueous solution of IL-18R in Example 1-1. The fractions corresponding to this single peak were collected, pooled and lyophilized, thus obtaining a purified IL-18R in solid form.

Thereafter, a portion of the purified IL-18R was sampled, incubated in PBS at 100°C for 5 minutes, and determined for residual activity by the method in Example 1-2, resulting in no binding to IL-18 which proved that IL-18R was inactivated by heating. This would support that the nature of this receptor is proteinaceous.

Further, a portion of the purified IL-18R obtained in the above was dissolved in an appropriate amount of PBS, dialyzed against PBS at ambient temperature overnight, added with an appropriate amount of ¹²⁵I-labelled human IL-18 prepared by the method in Example 1-1 and 1mM "BS³", a polymerizing agent commercialized by Pierce, Rockford, U.S.A., and allowed to stand at 0°C for 2 hours to form a conjugate of IL-18R and ¹²⁵I-labelled human IL-18. The reaction mixture was added with Tris-HCl buffer (pH7.5), allowed to stand at 0°C for an additional 1 hour to suspend the conjugation reaction, separated into respective proteinaceous components on SDS-PAGE using a set of molecular weight markers and dithiothreitol as reducing agent, and subjected to autoradiogram analysis.

The apparent molecular weight for this conjugate of IL-18R and ¹²⁵I-labelled human IL-18 was about 50,000 to 200,000 daltons when estimated with reference to the mobility of molecular weight markers on the autoradiogram. Since the molecular weight of IL-18 is about 20,000 daltons, the molecular weight of IL-18R can be estimated about 30,000-180,000 daltons on the assumption that IL-18R binds one human IL-18 molecule.

Example 1-6

Peptide mapping of IL-18R

A purified IL-18R obtained by the method in Example 1-5 was electrophoresed on SDS-PAGE using 7.5 w/v % gel with 2 w/v % dithiothreitol as reducing agent, and the gel was then soaked for 5 minutes in a mixture solution of 40 v/v % aqueous methanol and 1 v/v % acetic acid with 0.1 w/v % Coomassie Brilliant Blue for development, and soaked for an additional 2 hours for destaining in the same solution but without Coomassie Brilliant Blue, after which the stained part in the gel, molecular weight of 80,000-110,000 daltons, was cut off, added with 50 v/v % aqueous acetonitrile containing 0.2 M (NH₄)₂CO₃ and repeatedly agitated at ambient temperature. Thereafter, the gel slices were lyophilized, added with 0.2M (NH₄)₂CO₃ (pH 8.0), allowed to stand for 5 minutes to effect swelling, added with appropriate amounts of 1mM hydrochloric acid with 0.1μg/μl "SEQUENCING GRADE MODIFIED TRYPSIN", a reagent of trypsin commer-

cialized by Promega Corp., Madison, U.S.A., and 0.2 M $(\text{NH}_4)_2\text{CO}_3$ (pH 8.9), and allowed to react at 37°C overnight. After suspending with 10 v/v % aqueous acetic acid solution, the reaction mixture was added with a mixture solution of 0.1 v/v % trifluoroacetic acid and 60 v/v % aqueous acetonitrile and agitated at ambient temperature, after which the resultant supernatant was collected, concentrated *in vacuo* and centrifugally filtered, thus obtaining a concentrate with peptide fragments.

The concentrate was charged to "μRPC C2/C18 SC2.1/10", a column for high-performance liquid chromatography commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, pre-equilibrated with 0.065 v/v % trifluoroacetic acid, and then applied at a flow rate of 100μl/min with 0.055 v/v % trifluoroacetic acid containing 80 v/v % aqueous acetonitrile under liner gradient of acetonitrile increasing from 0 to 80 v/v % over 160 minutes immediately after application of the eluent. While monitoring the absorbance at a wavelength of 240nm, the eluate was fractioned to separately collect respective peptide fragments which eluted about 45, 50, 55, 58, 62, 72, 75 and 77 minutes after application of the eluent. The peptide fragments (hereinafter referred to as "peptide fragment 1", "peptide fragment 2", "peptide fragment 3", "peptide fragment 4", "peptide fragment 5", "peptide fragment 6", "peptide fragment 7" and "peptide fragment 8" in the order of elution) were analyzed in usual manner for amino acid sequence using "MODEL 473A", a protein sequencer commercialized by Perkin-Elmer Corp., Norwalk, U.S.A, revealing that the peptide fragments 1 to 8 bore the amino acid sequences of SEQ ID NOs:12 to 19 respectively. The peptide map obtained by this operation was as shown in FIG.5.

Example 2

Preparation of DNA

Example 2-1

Preparation of total RNA

In usual manner, L428 cells (FERM BP-5777) were suspended in RPMI-1640 medium (pH7.2) supplemented with 10 v/v % fetal bovine serum, and proliferated at 37°C while scaling up the cultivation. When the cell density reached a prescribed level, the proliferated cells were collected, suspended in 10mM sodium citrate (pH7.0) containing both 6M guanidine isothiocyanate and 0.5 w/v% sodium N-laurylsarcosinate, and then disrupted with a homogenizer.

Aliquots of 0.1M EDTA (pH 7.5) containing 5.7M CsCl_2 were placed in 35ml-reaction tubes, poured with the cell disruptant obtained in the above in layer over the EDTA in each tube, and subjected to ultracentrifugation at 20°C at 25,000rpm for 20 hours to collect the RNA fraction. The RNA fraction was distributed in 15ml-centrifugation tubes, added with an equivolume each of a mixture solution of chloroform/1-butanol (volume ratio 4:1), agitated for 5 minutes and centrifuged at 4°C at 10,000rpm for 10 minutes, after which the aqueous layer was collected, added with 2.5-fold volume of ethanol and allowed to stand at 20°C for 2 hours to precipitate the total RNA. The precipitate was collected, washed with 75 v/v % aqueous ethanol, and then dissolved in 0.5ml of sterilized distilled water to obtain a solution of the total RNA originating from L428 cell.

Example 2-2

Preparation of mRNA

An aqueous solution containing total RNA solution obtained by the method in Example 2-1 was added with 0.5ml of 10mM Tris-HCl buffer (pH 7.5), containing both 1mM EDTA and 0.1 w/v % sodium N-laurylsarcosinate, to bring the total volume to 1 ml. The mixture solution was added with 1 ml of "OLIGOTEX™-dT30 (SUPER)", a latex with an oligonucleotide of $(\text{dT})_{30}$ commercialized by Japan Roche K. K., Tokyo, Japan, reacted at 65°C for 5 minutes and rapidly cooled in an ice-chilling bath. Thereafter, the reaction mixture was added with 0.2ml of 5mM NaCl, incubated at 37°C for 10 minutes, centrifuged at 10,000rpm for 10 minutes to collect the resultant precipitate in pellet form which was then suspended in 0.5ml of sterilized distilled water and incubated at 65°C for 5 minutes to desorb the mRNA from the latex. The obtained solution was added with an appropriate amount of ethanol, and the resultant precipitate was collected and lyophilized to obtain a solid of mRNA.

Example 2-3

Preparation of DNA fragment encoding polypeptide

Four microliters of 25mM MgCl_2 , 2μl of 100mM Tris-HCl buffer (pH 8.3) containing 500mM KCl, 1μl of 25mM dNTP

mix, 0.5 µl of 40units/µl ribonuclease inhibitor and 1µl of 200units/µl reverse transcriptase were placed in a 0.5ml-reaction tube, added with 10 ng of an mRNA, obtained by the method in Example 2-2, along with an appropriate amount of random hexanucleotides, and added with sterilized distilled water to bring the total volume of 20µl. The obtained mixture was incubated first at 42°C for 20 minutes, then at 99°C for 5 minutes to suspend the reaction, thus obtaining a reaction mixture containing a first strand cDNA.

Twenty microliters of the reaction mixture was added with 1µl of 2.5 units/µl "CLONED Pfu POLYMERASE", a DNA polymerase commercialized by Stratagene Cloning Systems, California, U.S.A., 10µl of the reaction buffer and 1µl of 25mM dNTP mix, both commercialized by Stratagene Cloning Systems, added with 0.1µg each of oligonucleotides as sense and antisense primers having respective nucleotide sequences as shown with 5'-TCAGTCGACGCCACCAT-GAATTGTAGAGAA-3' and 5'-GAAGCGGCCGCATCATTAACTCGGAAAGAAC-3' which had been prepared on the basis of the amino acid sequence described in P. Parneet et al., *The Journal of Biological Chemistry*, Vol.271, pp.3967-3970 (1996), added with sterile distilled water to bring the total volume to 100µl. The resultant mixture was subjected first to 3-time cycles of incubating at 95°C for 1 minute, 42°C for 2 minutes and 72°C for 3 minutes in the given order, then to 35-time cycles of incubating at 95°C for 1 minute, 60°C for 2 minutes and 72°C for 3 minutes in the given order to effect PCR reaction.

Fifty nanograms of the obtained PCR product was added with 1 ng of "pCR-Script Cam SK(+)", a plasmid vector commercialized by Stratagene Cloning Systems, California, U.S.A., and then subjected to ligation reaction at 16°C for 2 hours using "DNA LIGATION KIT VERSION 2", a DNA ligation kit commercialized by Takara Syuzo, Co., Ltd., Otsu, Shiga, Japan, to insert the DNA fragment of the PCR product in the plasmid vector. A portion of the reaction product was sampled and used in usual manner to transform "XL1-BLUE MRF' KAN", an *Escherichia coli* strain commercialized by Stratagene Cloning Systems, California, U.S.A.

Example 3

Preparation of recombinant DNA

A transformant obtained by the method in Example 2-3 was inoculated in LB medium containing 30µg/ml chloramphenicol and cultivated at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. After confirming by the dideoxy method that the plasmid DNA contained the nucleotide sequence of SEQ ID NO:7, the plasmid DNA was exposed to both restriction enzymes *NotI* and *SaII*, and 100 ng of the obtained DNA fragment was added with 10ng of "pcDNA1/Amp", a plasmid vector with a modified multiple cloning site, commercialized by Invitrogen Corporation, San Diego, U.S.A., which had been predigested with both restriction enzymes *NotI* and *XhoI*, and subjected to ligation reaction at 16°C for 2 hours using "LIGATION KIT VERSION 2", a ligation kit commercialized by Takara Syuzo Co., Ltd., Otsu, Shiga, Japan. A portion of the reaction product was sampled and introduced in usual manner into "XL1-BLUE MRF' KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., to obtain a transformant "cDNA/HuIL-18R" which contained a recombinant DNA "pcDNA/HuIL-18R" of this invention. The recombinant DNA "pcDNA/HuIL-18R" was analyzed in usual manner, revealing that in the recombinant DNA, a DNA "IL-18R cDNA", which contained the nucleotide sequence of SEQ ID NO:1 encoding the polypeptide of this invention, was linked downstream the cytomegalo virus promotor Pcmv, as shown in FIG. 6.

Example 4

Preparation of transformant

A transformant "cDNA/HuIL-18R" obtained by the method in Example 3 was inoculated in LB medium (pH 7.5) containing 100µg/ml ampicillin and cultured at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. Separately, COS-1 cell (ATCC CRL-1650), a fibroblastic cell line derived from a kidney of African green monkey was proliferated in usual manner, and 20 micrograms of the plasmid DNA obtained in the above was introduced by conventional electroporation method into 1×10^7 COS-1 cells to obtain transformant cells which contained the DNA of this invention.

Example 5

Preparation of polypeptide

DMEM medium (pH 7.2) supplemented with 10 v/v % fetal bovine serum was distributed in flat-bottomed culture bottles, inoculated with transformant cells, obtained by the method in Example 4, to give a cell density of 1×10^5

cells/ml, and cultured at 37°C in 5 v/v % CO₂ incubator for 3 days. After removing the supernatant from the culture, PBS containing both 5mM EDTA and 0.02 w/v % NaN₃ was placed in the culture bottles to desorb the proliferated cells.

After washing in PBS, the proliferated cells were rinsed in a buffer containing 20mM HEPES, 10mM KCl, 1.5mM MgCl₂ and 0.1mM EDTA (hereinafter referred to as "hypotonic buffer"), and suspended in a fresh preparation of the hypotonic buffer to give a cell density of 2×10^7 cells/ml. The cell suspension was homogenized with a Dounce-type homogenizer under ice-chilling conditions, and the resultant homogenate was centrifuged at 15,000rpm at 5 minutes to remove both cell nuclei and intact cells, and dialyzed overnight against PBS containing 2mM CHAPS.

The dialyzed product was charged to a column of immobilized monoclonal antibody MAb #117-10C, prepared by the method in Example 1-5, which was then applied with PBS containing 12mM CHAPS to remove non-adsorbed components. Thereafter, the column was applied with 35 mM ethylamine (pH10.8) containing 2 mM CHAPS while collecting and fractionating the eluate. was applied to the column, and the eluate was fractionally collected. Each fraction was then checked for presence of the polypeptide of human origin by the method in Example 1-1 using ¹²⁵I-labelled human IL-18, selected and pooled to obtain per 10⁸ starting cells about 2 ml of an aqueous solution which contained a polypeptide with the amino acid sequence of SEQ ID NO:20. The protein content in the solution was about 10µg/ml.

The polypeptide thus obtained was studied for physicochemical properties by the methods in Example 1. As the result, the polypeptide obtained in this Example contained each amino acid sequence in SEQ ID NOs:12 to 19 as partial amino acid sequences, as well as exhibiting physiological activities which were similar to those of the IL-18R from L428 cell.

Example 6

Soluble polypeptide from human origin

Example 6-1

Preparation of recombinant DNA

One nanogram of a recombinant DNA "pcDNA/HuIL-18R" obtained by the method in Example 3, 10µl of 10×PCR buffer and 1µl of 25mM dNTP mix were placed in 0.5ml-reaction tube, added with 1 microliter of 2. units/microliter Pfu DNA polymerase, added with appropriate amounts of oligonucleotides as sense and antisense primers having respective nucleotide sequences as shown with 5'-TCAGTCGACGCCACCATGAATTGTAGAGAATTA-3' and 5'-GAAGCGGCCGCATCATTATCTTGTGAAGACGTG-3', and with sterile distilled water to bring the total volume to 100µl. The resultant mixture was subjected first to 3-time cycles of incubating at 94°C for 1 minute, 42°C for 2 minutes and 72°C for 3 minutes in the given order, then to 35-time cycles of incubating at 94°C for 1 minute, 60°C for 2 minutes and 72°C for 3 minutes in the given order to effect PCR reaction.

Fifty nanograms of the obtained PCR product was added with 1ng of "pCR-SCRIPT SK(+)", a plasmid vector commercialized by Takara Syuzo Co. Ltd., Otsu, Shiga, Japan, and reacted using "DNA LIGATION KIT VERSION 2", a DNA ligation kit commercialized by Takara Shuzo Co. Ltd., Otsu, Shiga, Japan, at 16°C for 2 hours to insert the DNA fragment as the PCR product into the plasmid vector. A portion of the reaction product was sampled and "XL1-BLUE MRF' KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., was transformed therewith in usual manner.

The transformant obtained in the above was inoculated in LB medium (pH 7.5) containing 100µg/ml ampicillin and cultivated at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. After confirming by the dideoxy method that the plasmid DNA contained the nucleotide sequence of SEQ ID NO:10, the plasmid DNA was exposed to both restriction enzymes *NotI* and *SalI*, and 100 ng of the resultant DNA fragment was added with 10ng of "pEF-BOS", a plasmid vector prepared in accordance with the method described in S. Mizushima, *Nucleic Acid Research*, Vol.18, No.17, pp.5,332 (1990) with slight modification and also predigested with both restriction enzymes *NotI* and *XhoI*, and subjected to ligation reaction using "LIGATION KIT VERSION 2", a DNA ligation kit commercialized by Takara Shuzo Co., Ltd., Otsu, Shiga, Japan, at 16°C for 2 hours. A portion of the reaction product was sampled and introduced in usual manner into "XL1-BLUE MRF' KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., thus obtaining a transformant "EFHIL18R-14" which contained a recombinant DNA "pEFHIL18R-14" of this invention. The recombinant DNA "pEFHIL18R-14" was analyzed in usual manner, revealing that in the recombinant DNA, a cDNA "EFHIL18R-14 cDNA", which contained the nucleotide sequence of SEQ ID NO:6 encoding the polypeptide of this invention, was located downstream the elongation factor 1 promotor EF1αP as shown in FIG. 7.

Example 6-2Preparation of transformant

A transformant "EFHIL18R-14" obtained by the method in Example 6-1 was inoculated in LB medium (pH 7.5) containing 100µg/ml ampicillin and cultivated at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. Separately, COS-1 cell (ATCC CRL-1650), a fibroblastoid cell line derived from a kidney of African green monkey, was proliferated in usual manner, and 20 micrograms of the plasmid DNA obtained in the above was introduced by conventional electroporation method into 1×10^7 COS-1 cells to obtain transformant cells which contained the DNA of this invention.

Example 6-3Preparation of soluble polypeptide

"ASF104", a serum-free nutrient culture medium commercialized by Ajinomoto Co., Inc., Tokyo, Japan, was distributed in flat-bottomed culture bottles, inoculated with transformant cells, obtained by the method in Example 6-2, to give a cell density of 1×10^5 cells/ml, and cultured in usual manner at 37°C in 5 v/v % CO₂ incubator for 3 days. The supernatant was collected from the culture and charged to a column of an immobilized monoclonal antibody #117-10C prepared by the method in Example 1-5, after which the column was applied first with PBS containing 12mM CHAPS to remove non-adsorbed components, then with 35mM ethylamine (pH 10.8) containing 2 mM CHAPS while collecting and fractionating the eluate. Each fraction was checked for presence of human soluble polypeptide by the method in Example 1-1 using ¹²⁵I-labelled human IL-18, selected and pooled to obtain per 10⁸ starting cells about 2 ml of an aqueous solution which contained a polypeptide with the amino acid sequence of SEQ ID NO:22. The protein content in the solution was about 10µg/ml.

The soluble polypeptide thus obtained was studied for physicochemical properties by the method in Example 1. As the result, the soluble polypeptide obtained in this Example contained each amino acid sequences in SEQ ID NOs:12 to 17 and 19 as partial sequences, as well as exhibiting physiological activities which were similar to the IL-18R from L428 cell.

Example 7Soluble polypeptide of human origin

One nanogram of a recombinant DNA "pEFHIL18R-14" obtained by the method in Example 6-1, 10µl of 10xPCR buffer and 1µl of 25mM dNTP mix were placed in 0.5ml-reaction tube, added with 1µl of 2.5units/µl Pfu DNA polymerase, further added with appropriate amounts of oligonucleotides as sense and antisense primers having respective nucleotide sequences as shown with 5'-TCAGTCGACGCCACCATGAATTGTAGAG-3' and 5'-GAAGCGGCCGCTCATTAGTGATGGTGATGTGCAACATGGTTAAGCTT-3', and filled up to 100µl with sterile distilled water. The resultant mixture was subjected first to 3-time cycles of incubating at 94°C for 1 minute, 42°C for 2 minutes and 72°C for 1 minute in the given order, then to 35-time cycles of incubating at 94°C for 1 minute, 64°C for 1 minute and 72°C for 1 minute in the given order to effect PCR reaction, thus obtaining a DNA fragment which consisted of the nucleotide sequence of SEQ ID NO:5, a digestion site for restriction enzyme *Sa*I and a Kozak's sequence both linked to the 5'-terminal of the nucleotide sequence of SEQ ID NO:5, and a digestion site for restriction enzyme *Not*I and a nucleotide sequence encoding (His)₆ tag both linked to the 3'-terminal of the nucleotide sequence of SEQ ID NO:5. This DNA fragment was introduced similarly as in Example 6-1 in "XL1-Blue MRF Kan", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., to obtain a transformant which contained a recombinant DNA "pEFHIL18RD1-2-H" according to this invention. Analysis of the recombinant DNA in usual manner confirmed that in this recombinant DNA a cDNA "HIL18RD1-2-H", which contained the nucleotide sequence of SEQ ID NO:5 encoding the polypeptide of this invention, was located downstream the elongation factor promoter EF1αP as shown in FIG. 8.

The recombinant DNA "pEFHIL18RD1-2-H" was introduced in COS-1 cells similarly as in Example 6-2 using the transformant thus obtained, and the COS-1 cells were then cultivated similarly as in Example 6-3. The supernatant of the resultant culture was concentrated with membrane filtration, and charged on a column of "Ni-NTA Spin Kit", a gel product for affinity chromatography commercialized by QIAGEN GmbH, Hilden, Germany, which was then applied with PBS containing 20mM imidazole to remove the non-adsorbed fractions. Thereafter, the column was applied with PBS containing 250mM imidazole, and the eluate was collected in fractions while checking the presence of human soluble polypeptide in each fraction by the method in Example 1-1 using ¹²⁵I-labelled human IL-18, after which the fractions

with the polypeptide were collected and pooled, thus obtaining about 2ml of an aqueous solution containing the polypeptide with the amino acid sequence of SEQ ID NO:23 per starting 10^8 cells. The protein content in the solution was about 10µg/ml.

The soluble polypeptide thus obtained was studied for physicochemical properties by the method in Example 1. As the result, the soluble polypeptide obtained in this Example contained a part or whole of each amino acid sequences in SEQ ID NOs:14 to 16 and 19 as partial amino acid sequences, as well as exhibiting physiological activities which were similar to those of IL-18R from L428 cell.

Example 8

Soluble polypeptide of human origin

A transformant containing a recombinant DNA "pEFHIL18RD1-H" according to this invention was prepared similarly as in Example 7, except that sense and antisense primers were replaced with oligonucleotides having respective nucleotide sequences as shown with 5'-TCAGTCGACGCCACCATGAATTGTAGAG-3' and GAAGCGGCCGCT-CATTAGTGATGGTGATGGTGATGTCTTTCAGTGAAACAGCT-3'. Analysis of the recombinant DNA in usual manner confirmed that in the recombinant DNA a cDNA "HIL18RD1-H", which contained the nucleotide sequence of SEQ ID NO:3 encoding the polypeptide of this invention, was located downstream the elongation factor promotor EF1αP as shown in FIG. 9. Thereafter, similarly as in Example 7, the recombinant DNA was introduced in COS-1 cells and brought into expression, thus obtaining about 2ml of an aqueous solution containing a polypeptide with the amino acid sequence of SEQ ID NO:24 per 10^8 starting cells. The protein content in the solution was about 10µg/ml.

The polypeptide of this invention thus obtained were studied for physicochemical properties by the method in Example 1. As the result, the soluble polypeptide obtained in this Example contained each amino acid sequences of SEQ ID NOs:14 and 15 as partial amino acid sequences, as well as exhibiting physiological activities which were similar to those of the IL-18R from L428 cell.

Example 9

Soluble polypeptide of mouse origin

Example 9-1

Preparation of recombinant DNA

A reaction product containing a first strand cDNA was obtained by subjecting an mRNA, prepared in usual manner from mouse liver, in place with that from L428 cell to the same reaction to synthesize first strand cDNA as in Example 2-3. The reaction product was treated by the same PCR method as in Example 2-3, except that the sense and antisense primers were replaced with oligonucleotides having respective nucleotide sequence as shown with 5'-TCAGTCGACGCCACCATGCATCATGAAGAA-3' and 5'-GAAGCGGCCGCGCATCATTAGTGATGGTGAT-GGTGATGTGTAAGACATGGCC-3', which had been prepared on the basis of the amino acid sequence described in P. Parnet et al., *The Journal of Biological Chemistry*, Vol.271, pp.3,967-3,970 (1996) and also the nucleotide sequence of SEQ ID NO:1. This operation gave a DNA fragment which comprised the nucleotide sequence of SEQ ID NO:11, a digestion site for restriction enzyme *Sall* linked to the 5'-terminal in the nucleotide sequence of the SEQ ID NO:11, and a cleavage site for restriction enzyme *NotI* and a nucleotide sequence encoding (His)₆ tag both linked to the 3'-terminal in the nucleotide sequence of the SEQ ID NO:11.

According to the method in Example 6-1, this DNA fragment was introduced into "XL1-BLUE MRF' KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., to transform. After a plasmid DNA was collected from the transformant and confirmed to contain the nucleotide sequence of SEQ ID NO:11, the plasmid DNA was introduced into "XL1-BLUE MRF' KAN", a strain of *Escherichia coli* strain commercialized by Stratagene Cloning Systems, California, U.S.A., to obtain a transformant "EFMIL18RSHT" which contains a recombinant DNA "pEFMIL18RSHT" according to this invention. Analysis in usual manner confirmed that in the recombinant DNA "pEFMIL18RSHT" a cDNA "EFMIL18RSHT cDNA", which contained the nucleotide sequence of SEQ ID NO:4 encoding the polypeptide of this invention, was linked to downstream of the elongation factor 1 promotor EF1αP, as shown in FIG. 8.

Example 9-2Preparation of transformant and soluble polypeptide

According to the method in Example 6-2, a plasmid DNA was collected from a transformant "EFMIL18RSHT" obtained by the method in Example 9-1, and introduced into COS-1 cells to obtain transformant cells which contained a DNA encoding a soluble polypeptide of mouse origin.

"ASF104", a serum-free nutrient culture medium commercialized by Ajinomoto Co., Inc., Tokyo, Japan, was distributed in flat-bottomed culture bottles, inoculated with the transformed COS-1 cells to give a cell density of 1×10^5 cells/ml, and cultivated in usual manner at 37°C in 5 v/v % CO₂ incubator for 3 days. The supernatant was collected from the resultant culture and charged to a column of "Ni-NTA", a gel product for affinity chromatography, commercialized by QIAGEN GmbH, Hilden, Germany, after which the column was applied first with PBS containing 20mM imidazole to remove non-adsorbed components, then with PBS containing 250mM imidazole while collecting and fractionating the eluate. The fractions were checked for presence of mouse soluble polypeptide by the method in Example 1-1 using ¹²⁵I-labelled mouse IL-18, selected and pooled, thus obtaining per 10⁸ starting cells about 2 ml of an aqueous solution which contained a polypeptide with the amino acid sequence of SEQ ID NO:25. The protein content in the solution was about 100µg/ml. The soluble polypeptide thus obtained was studied in accordance with the method in Example 1, revealing that it efficiently neutralized mouse IL-18.

Example 10Liquid agent

Either polypeptide obtained by the method in Examples 5 to 8 was separately dissolved in aliquots of physiological saline containing as stabilizer 1 w/v % "TREHAOSE", a powdered crystalline trehalose commercialized by Hayashibara Co., Ltd., Okayama, Japan, to give respective concentration of 1 mg/ml, and the resultant mixtures were separately and sterilely filtered with membrane in usual manner to obtain four distinct liquid agents.

The products, which are excellent in stability, are useful as injection, ophthalmic solution and collunarium in treatment and prevention of susceptible diseases including autoimmune diseases.

Example 11Dried injection

One hundred milligrams of either polypeptide obtained by the methods in Example 5 to 8 was separately dissolved in aliquots of physiological saline containing 1 w/v % sucrose as stabilizer, the resultant solutions were separately and sterilely filtered with membrane, distributed in vials in every 1 ml aliquot, lyophilized and sealed in usual manner to obtain four distinct pulverized agents.

The products, which are excellent in stability, are useful as dried injection in treatment and prevention of susceptible diseases including autoimmune diseases.

Example 12Ointment

"HI-BIS-WAKO 104", a carboxyvinylpolymer commercialized by Wako Pure Chemicals, Tokyo, Japan, and "TREHAOSE", a powdered crystalline trehalose commercialized by Hayashibara Co., Ltd., Okayama, Japan, were dissolved in sterilized distilled water to give respective concentrations of 1.4 w/w % and 2.0 w/w %, and either polypeptide obtained by the methods in Examples 5 to 8 was separately mixed with aliquots of the resultant solution to homogeneity, and adjusted to pH7.2 to obtain four distinct paste agents containing about 1 mg/g of the polypeptide of this invention each.

The products, which are excellent in both spreadability and stability, are useful as ointment in treatment and prevention of susceptible diseases including autoimmune diseases.

Example 13Tablet

Aliquots of "FINETOSE", a pulverized anhydrous crystalline alpha-maltose commercialized by Hayashibara Co., Ltd., Okayama, Japan, were separately admixed with either polypeptide, obtained by the methods in Examples 5 to 8, and aliquots of "LUMIN" as cell activator, [bis-4-(1-ethylquinoline)][γ -4'-(1-ethylquinoline)] pentamethionine cyanine, to homogeneity, and the resultant mixtures were separately tableted in usual manner to obtain four distinct types of tablets, about 200 mg each, containing about 1mg/tablet of the polypeptide of this invention and also 1mg/tablet of LUMIN each.

The products, which are excellent in swallowability and stability and also bears an cell activating property, are useful as tablet in treatment and prevention of susceptible diseases including autoimmune diseases.

ExperimentAcute toxicity test

In usual manner, a variety of agents, obtained by the methods in Examples 8 to 11, were percutaneously or orally administrated or intraperitoneally injected to 8 week-old mice. As the result, the LD₅₀ of each sample was proved about 1 mg or higher per body weight of mouse in terms of the amount of the polypeptide, regardless of administration route. This does support that the polypeptide of this invention is safe when incorporated in pharmaceuticals directed to use in mammals including human.

As explained above, this invention is based on the discovery of a novel receptor protein which recognizes IL-18. The polypeptide of this invention exhibits a remarkable efficacy in relief of rejection reaction associated with grafts of organs and also in treatment and prevention of various disease resulting from excessive immunoreaction because the polypeptide bears properties of suppressing and regulating immunoreaction in mammals including human. Further, the polypeptide of this invention is useful in clarification of physiological activities of IL-18, establishment of hybridoma cells which are capable of producing monoclonal antibodies specific to IL-18R, and also affinity chromatography and labelled assay to purify and detect IL-18. In addition, the polypeptide of this invention, in particular, that in soluble form is useful in screening *in vivo* and *in vitro* agonists and antagonists to IL-18. The polypeptide of this invention, which bears these outstanding usefulness, can be easily prepared in desired amounts by the process according to this invention using recombinant DNA techniques.

This invention, which exhibits these remarkable effects, would be very significant and contributive to the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

NAME:KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU
KENKYUJO

(ii) TITLE OF INVENTION:POLYPEPTIDES

(iii) NUMBER OF SEQUENCES:27

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(A) ADDRESSEE:KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU
KAGAKU KENKYUJO

(B) STREET:2-3, 1-CHOME, SHIMOISHII

(C) CITY:OKAYAMA

(E) COUNTRY:JAPAN

(F) POSTAL CODE (ZIP):700

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:Floppy disk

(B) COMPUTER:IBM PC compatible

(C) OPERATING SYSTEM:PC-DOS/MS-DOS

(vii) PRIOR APPLICATION DATA:

(A1) APPLICATION NUMBER: JP 74,697/97

(B1) FILING DATE:March 12, 1997

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(A2) APPLICATION NUMBER: JP 215,488/97

(B2) FILING DATE:July 28, 1997

(vii) PRIOR APPLICATION DATA:

(A3) APPLICATION NUMBER: JP 291,837/97

(B3) FILING DATE:October 9, 1997

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1563 base pairs

(B) TYPE:nucleic acid

(C) strandedness:double

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(ix) FEATURE:

(A) NAME/KEY:mat peptide

(B) LOCATION:1..1563

(C) IDENTIFICATION METHOD:E

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:1:

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96
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
20 25 30
ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG
144
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu

EP 0 864 585 A1

			35				40				45						
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5	192	Leu	Asn	Pro	Arg	Ser	Ser	Ser	Arg	Ile	Ala	Leu	His	Asp	Cys	Val	Leu
			50				55					60					
	GAG	TTT	TGG	CCA	GTT	GAG	TTG	AAT	GAC	ACA	GGA	TCT	TAC	TTT	TTC	CAA	
	240	Glu	Phe	Trp	Pro	Val	Glu	Leu	Asn	Asp	Thr	Gly	Ser	Tyr	Phe	Phe	Gln
		65				70					75					80	
10	ATG	AAA	AAT	TAT	ACT	CAG	AAA	TGG	AAA	TTA	AAT	GTC	ATC	AGA	AGA	AAT	
	288	Met	Lys	Asn	Tyr	Thr	Gln	Lys	Trp	Lys	Leu	Asn	Val	Ile	Arg	Arg	Asn
					85						90				95		
	AAA	CAC	AGC	TGT	TTC	ACT	GAA	AGA	CAA	GTA	ACT	AGT	AAA	ATT	GTG	GAA	
	336	Lys	His	Ser	Cys	Phe	Thr	Glu	Arg	Gln	Val	Thr	Ser	Lys	Ile	Val	Glu
				100					105					110			
	GTT	AAA	AAA	TTT	TTT	CAG	ATA	ACC	TGT	GAA	AAC	AGT	TAC	TAT	CAA	ACA	
	384	Val	Lys	Lys	Phe	Phe	Gln	Ile	Thr	Cys	Glu	Asn	Ser	Tyr	Tyr	Gln	Thr
			115				120					125					
20	CTG	GTC	AAC	AGC	ACA	TCA	TTG	TAT	AAG	AAC	TGT	AAA	AAG	CTA	CTA	CTG	
	432	Leu	Val	Asn	Ser	Thr	Ser	Leu	Tyr	Lys	Asn	Cys	Lys	Lys	Leu	Leu	Leu
			130				135				140						
	GAG	AAC	AAT	AAA	AAC	CCA	ACG	ATA	AAG	AAG	AAC	GCC	GAG	TTT	GAA	GAT	
	480	Glu	Asn	Asn	Lys	Asn	Pro	Thr	Ile	Lys	Lys	Asn	Ala	Glu	Phe	Glu	Asp
		145				150					155					160	
	CAG	GGG	TAT	TAC	TCC	TGC	GTG	CAT	TTC	CTT	CAT	CAT	AAT	GGA	AAA	CTA	
	528	Gln	Gly	Tyr	Tyr	Ser	Cys	Val	His	Phe	Leu	His	His	Asn	Gly	Lys	Leu
					165					170					175		
30	TTT	AAT	ATC	ACC	AAA	ACC	TTC	AAT	ATA	ACA	ATA	GTG	GAA	GAT	CGC	AGT	
	576	Phe	Asn	Ile	Thr	Lys	Thr	Phe	Asn	Ile	Thr	Ile	Val	Glu	Asp	Arg	Ser
				180				185					190				
	AAT	ATA	GTT	CCG	GTT	CTT	CTT	GGA	CCA	AAG	CTT	AAC	CAT	GTT	GCA	GTG	
	624	Asn	Ile	Val	Pro	Val	Leu	Leu	Gly	Pro	Lys	Leu	Asn	His	Val	Ala	Val
			195				200				205						
	GAA	TTA	GGA	AAA	AAC	GTA	AGG	CTC	AAC	TGC	TCT	GCT	TTG	CTG	AAT	GAA	
	672	Glu	Leu	Gly	Lys	Asn	Val	Arg	Leu	Asn	Cys	Ser	Ala	Leu	Leu	Asn	Glu
			210			215					220						
40	GAG	GAT	GTA	ATT	TAT	TGG	ATG	TTC	GGG	GAA	GAA	AAT	GGA	TCG	GAT	CCT	
	720	Glu	Asp	Val	Ile	Tyr	Trp	Met	Phe	Gly	Glu	Glu	Asn	Gly	Ser	Asp	Pro
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	AAT	ATA	CAT	GAA	GAG	AAA	GAA	ATG	AGA	ATT	ATG	ACT	CCA	GAA	GGC	AAA	
	768	Asn	Ile	His	Glu	Glu	Lys	Glu	Met	Arg	Ile	Met	Thr	Pro	Glu	Gly	Lys
					245				250						255		
	TGG	CAT	GCT	TCA	AAA	GTA	TTG	AGA	ATT	GAA	AAT	ATT	GGT	GAA	AGC	AAT	
	816	Trp	His	Ala	Ser	Lys	Val	Leu	Arg	Ile	Glu	Asn	Ile	Gly	Glu	Ser	Asn
				260			265				270						
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	864	Leu	Asn	Val	Leu	Tyr	Asn	Cys	Thr	Val	Ala	Ser	Thr	Gly	Gly	Thr	Asp
			275				280						285				

55

EP 0 864 585 A1

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912
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290 295 300
5 CAC GTC TTC ACA AGA GGA ATG ATC ATA GCT GTT TTG ATC TTG GTG GCA
960
His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu Ile Leu Val Ala
305 310 315 320
GTA GTG TGC CTA GTG ACT GTG TGT GTC ATT TAT AGA GTT GAC TTG GTT
1008
10 Val Val Cys Leu Val Thr Val Cys Val Ile Tyr Arg Val Asp Leu Val
325 330 335
CTA TTT TAT AGA CAT TTA ACG AGA AGA GAT GAA ACA TTA ACA GAT GGA
1056
Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr Leu Thr Asp Gly
340 345 350
15 AAA ACA TAT GAT GCT TTT GTG TCT TAC CTA AAA GAA TGC CGA CCT GAA
1104
Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys Arg Pro Glu
355 360 365
AAT GGA GAG GAG CAC ACC TTT GCT GTG GAG ATT TTG CCC AGG GTG TTG
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GAG AAA CAT TTT GGG TAT AAG TTA TGC ATA TTT GAA AGG GAT GTA GTG
1200
Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg Asp Val Val
385 390 395 400
25 CCT GGA GGA GCT GTT GTT GAT GAA ATC CAC TCA CTG ATA GAG AAA AGC
1248
Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu Ile Glu Lys Ser
405 410 415
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1296
30 Arg Arg Leu Ile Ile Val Leu Ser Lys Ser Tyr Met Ser Asn Glu Val
420 425 430
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1344
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435 440 445
35 ATT AAA ATA ATC TTA ATT GAA TTT ACA CCT GTT ACT GAC TTC ACA TTC
1392
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450 455 460
TTG CCC CAA TCA CTA AAG CTT TTG AAA TCT CAC AGA GTT CTG AAG TGG
1440
40 Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg Val Leu Lys Trp
465 470 475 480
AAG GCC GAT AAA TCT CTT TCT TAT AAC TCA AGG TTC TGG AAG AAC CTT
1488
Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe Trp Lys Asn Leu
485 490 495
45 CTT TAC TTA ATG CCT GCA AAA ACA GTC AAG CCA GGT AGA GAC GAA CCG
1536
Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly Arg Asp Glu Pro
500 505 510
GAA GTC TTG CCT GTT CTT TCC GAG TCT
1563
50 Glu Val Leu Pro Val Leu Ser Glu Ser
515 520

55

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1557 base pairs

(B) TYPE:nucleic acid

(C) strandedness:double

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(ix) FEATURE:

(A) NAME/KEY:mat peptide

(B) LOCATION:1..1557

(C) IDENTIFICATION METHOD:S

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

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96
Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His Arg Asn
20 25 30
GAA ACA GCC ACC ATG AGA TGG TTC AAA GGC AGT GCT TCA CAT GAG TAT
144
Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr
35 40 45
AGA GAG CTG AAC AAC AGA AGC TCG CCC AGA GTC ACT TTT CAT GAT CAC
192
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50 55 60
ACC TTG GAA TTC TGG CCA GTT GAG ATG GAG GAT GAG GGA ACG TAC ATT
240
Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile
65 70 75 80
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288
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85 90 95
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336
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100 105 110
GTT GAA GTT AAC AAA TCT CTG CAT ATC ACT TGT AAG AAT CCT AAC TAT
384
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115 120 125
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432
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130 135 140
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Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly
145 150 155 160
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528
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180 185 190

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EP 0 864 585 A1

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 10 CTG TTT TAT TGG AGC ATC AGG AAA GAG GAC AGC TCA GAC CCT AAT GTG
 720
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 225 230 235 240
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 768
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 260 265 270
 20 AAT GTT TTA TAT AAT TGC ACC GTG GCC AAC GAA GAA GCC ATA GAC ACC
 864
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 275 280 285
 AAG AGC TTC GTC TTG GTG AGA AAA GAA ATA CCT GAT ATC CCA GGC CAT
 912
 25 Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His
 290 295 300
 GTC TTT ACA GGA GGA GTA ACT GTG CTT GTT CTC GCC TCT GTG GCA GCA
 960
 Val Phe Thr Gly Gly Val Thr Val Leu Val Leu Ala Ser Val Ala Ala
 305 310 315 320
 30 GTG TGT ATA GTG ATT TTG TGT GTC ATT TAT AAA GTT GAC TTG GTT CTG
 1008
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 TTC TAT AGG CGC ATA GCG GAA AGA GAC GAG ACA CTA ACA GAT GGT AAA
 1056
 35 Phe Tyr Arg Arg Ile Ala Glu Arg Asp Glu Thr Leu Thr Asp Gly Lys
 340 345 350
 ACA TAT GAT GCC TTT GTG TCT TAC CTG AAA GAG TGT CAT CCT GAG AAT
 1104
 Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys His Pro Glu Asn
 355 360 365
 40 AAA GAA GAG TAT ACT TTT GCT GTG GAG ACG TTA CCC AGG GTC CTG GAG
 1152
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 370 375 380
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 1200
 45 Lys Gln Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg Asp Val Val Pro
 385 390 395 400
 GGC GGA GCT GTT GTC GAG GAG ATC CAT TCA CTG ATA GAG AAA AGC CGG
 1248
 Gly Gly Ala Val Val Glu Glu Ile His Ser Leu Ile Glu Lys Ser Arg
 405 410 415
 50 AGG CTA ATC ATC GTT CTC AGC CAG AGT TAC CTG ACT AAC GGA GCC AGG
 1296
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 420 425 430
 CGT GAG CTC GAG AGT GGA CTC CAC GAA GCA CTG GTA GAG AGG AAG ATT

55

1344
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 435 440 445
 5 AAG ATC ATC TTA ATT GAG TTT ACT CCA GCC AGC AAC ATC ACC TTT CTC
 1392
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 CCC CCG TCG CTG AAA CTC CTG AAG TCC TAC AGA GTT CTA AAA TGG AGG
 1440
 10 Pro Pro Ser Leu Lys Leu Leu Lys Ser Tyr Arg Val Leu Lys Trp Arg
 465 470 475 480
 GCT GAC AGT CCC TCC ATG AAC TCA AGG TTC TGG AAG AAT CTT GTT TAC
 1488
 Ala Asp Ser Pro Ser Met Asn Ser Arg Phe Trp Lys Asn Leu Val Tyr
 485 490 495
 15 CTG ATG CCC GCA AAA GCC GTC AAG CCA TGG AGA GAG GAG TCG GAG GCG
 1536
 Leu Met Pro Ala Lys Ala Val Lys Pro Trp Arg Glu Glu Ser Glu Ala
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 CGG TCT GTT CTC TCA GCA CCT
 1557
 20 Arg Ser Val Leu Ser Ala Pro
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(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 312 base pairs
 (B) TYPE: nucleic acid
 (C) strandedness: double
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: cDNA
 (ix) FEATURE:
 (A) NAME/KEY: mat peptide
 (B) LOCATION: 1..312
 (C) IDENTIFICATION METHOD: S
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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 96
 Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
 20 25 30
 40 ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG
 144
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 35 40 45
 CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT TGT GTT TTG
 192
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 50 55 60
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 Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
 65 70 75 80
 50 ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC AGA AGA AAT
 288
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 85 90 95

55

AAA CAC AGC TGT TTC ACT GAA AGA
 312
 Lys His Ser Cys Phe Thr Glu Arg
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(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 921 base pairs
 (B) TYPE: nucleic acid
 (C) strandedness: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: mat peptide
 (B) LOCATION: 1..921
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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 Ser Lys Ser Cys Ile His Arg Ser Gln Ile His Val Val Glu Gly Glu
 1 5 10 15
 CCT TTT TAT CTG AAG CCA TGT GGC ATA TCT GCA CCA GTG CAC AGG AAT
 96
 Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His Arg Asn
 20 25 30
 GAA ACA GCC ACC ATG AGA TGG TTC AAA GGC AGT GCT TCA CAT GAG TAT
 144
 Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr
 35 40 45
 AGA GAG CTG AAC AAC AGA AGC TCG CCC AGA GTC ACT TTT CAT GAT CAC
 192
 Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His Asp His
 50 55 60
 ACC TTG GAA TTC TGG CCA GTT GAG ATG GAG GAT GAG GGA ACG TAC ATT
 240
 Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile
 65 70 75 80
 TCT CAA GTC GGA AAT GAT CGT CGC AAT TGG ACC TTA AAT GTC ACC AAA
 288
 Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val Thr Lys
 85 90 95
 AGA AAC AAA CAC AGC TGT TTC TCT GAC AAG CTC GTG ACA AGC AGA GAT
 336
 Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser Arg Asp
 100 105 110
 GTT GAA GTT AAC AAA TCT CTG CAT ATC ACT TGT AAG AAT CCT AAC TAT
 384
 Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro Asn Tyr
 115 120 125
 GAA GAG CTG ATC CAG GAC ACA TGG CTG TAT AAG AAC TGT AAG GAA ATA
 432
 Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys Glu Ile
 130 135 140
 TCC AAA ACC CCA AGG ATC CTG AAG GAT GCC GAG TTT GGA GAT GAG GGC
 480
 Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly
 145 150 155 160
 TAC TAC TCC TGC GTG TTT TCT GTC CAC CAT AAT GGG ACA CGG TAC AAC
 528
 Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg Tyr Asn

EP 0 864 585 A1

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165      170      175
ATC ACC AAG ACT GTC AAT ATA ACA GTT ATT GAA GGA AGG AGT AAA GTA
576
5  Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser Lys Val
    180      185      190
ACT CCA GCT ATT TTA GGA CCA AAG TGT GAG AAG GTT GGT GTA GAA CTA
624
Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val Glu Leu
    195      200      205
10 GGA AAG GAT GTG GAG TTG AAC TGC AGT GCT TCA TTG AAT AAA GAC GAT
672
Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys Asp Asp
    210      215      220
CTG TTT TAT TGG AGC ATC AGG AAA GAG GAC AGC TCA GAC CCT AAT GTG
720
15 Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro Asn Val
    225      230      235      240
CAA GAA GAC AGG AAG GAG ACG ACA ACA TGG ATT TCT GAA GGC AAA CTG
768
Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly Lys Leu
    245      250      255
20 CAT GCT TCA AAA ATA CTG AGA TTT CAG AAA ATT ACT GAA AAC TAT CTC
816
His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn Tyr Leu
    260      265      270
AAT GTT TTA TAT AAT TGC ACC GTG GCC AAC GAA GAA GCC ATA GAC ACC
864
25 Asn Val Leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile Asp Thr
    275      280      285
AAG AGC TTC GTC TTG GTG AGA AAA GAA ATA CCT GAT ATC CCA GGC CAT
912
Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His
    290      295      300
30 GTC TTT ACA
921
Val Phe Thr
305

```

(6) INFORMATION FOR SEQ ID NO:5:

- ```

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH:621 base pairs
 (B) TYPE:nucleic acid
 (C) strandedness:double
 (D) TOPOLOGY:linear
40 (ii) MOLECULE TYPE:cDNA
 (ix) FEATURE:
 (A) NAME/KEY:mat peptide
 (B) LOCATION:1..621
 (C) IDENTIFICATION METHOD:S
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

```

```

45 GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA GGG GAA CCT
48
Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro
1 5 10 15
TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG ATT GAA ACA
96
50 Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
 20 25 30
ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG
144

```

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EP 0 864 585 A1

Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu  
 35 40 45  
 CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT TGT GTT TTG  
 5 192  
 Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu  
 50 55 60  
 GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC TTT TTC CAA  
 240  
 Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln  
 65 70 75 80  
 10 ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC AGA AGA AAT  
 288  
 Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn  
 85 90 95  
 15 AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA ATT GTG GAA  
 336  
 Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu  
 100 105 110  
 GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC TAT CAA ACA  
 384  
 Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr  
 115 120 125  
 20 CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG CTA CTA CTG  
 432  
 Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu  
 130 135 140  
 25 GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG TTT GAA GAT  
 480  
 Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp  
 145 150 155 160  
 GAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT GGA AAA CTA  
 528  
 Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu  
 165 170 175  
 30 TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA GAT CGC AGT  
 576  
 Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser  
 180 185 190  
 35 AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT GTT GCA  
 621  
 Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala  
 195 200 205

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
 40 (A) LENGTH: 927 base pairs  
 (B) TYPE: nucleic acid  
 (C) strandedness: double  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: cDNA  
 (ix) FEATURE:  
 45 (A) NAME/KEY: mat peptide  
 (B) LOCATION: 1..927  
 (C) IDENTIFICATION METHOD: E  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA GGG GAA CCT  
 50 48  
 Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro  
 1 5 10 15  
 TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG ATT GAA ACA

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EP 0 864 585 A1

96  
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr  
20 25 30  
5 ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG  
144  
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu  
35 40 45  
CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT TGT GTT TTG  
192  
10 Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu  
50 55 60  
GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC TTT TTC CAA  
240  
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln  
65 70 75 80  
15 ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC AGA AGA AAT  
288  
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn  
85 90 95  
AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA ATT GTG GAA  
336  
20 Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu  
100 105 110  
GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC TAT CAA ACA  
384  
Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr  
115 120 125  
25 CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG CTA CTA CTG  
432  
Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu  
130 135 140  
GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG TTT GAA GAT  
480  
30 Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp  
145 150 155 160  
CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT GGA AAA CTA  
528  
Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu  
165 170 175  
35 TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA GAT CGC AGT  
576  
Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser  
180 185 190  
AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT GTT GCA GTG  
624  
40 Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala Val  
195 200 205  
GAA TTA GGA AAA AAC GTA AGG CTC AAC TGC TCT GCT TTG CTG AAT GAA  
672  
Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu Leu Asn Glu  
210 215 220  
45 GAG GAT GTA ATT TAT TGG ATG TTC GGG GAA GAA AAT GGA TCG GAT CCT  
720  
Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly Ser Asp Pro  
225 230 235 240  
AAT ATA CAT GAA GAG AAA GAA ATG AGA ATT ATG ACT CCA GAA GGC AAA  
768  
50 Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro Glu Gly Lys  
245 250 255  
TGG CAT GCT TCA AAA GTA TTG AGA ATT GAA AAT ATT GGT GAA AGC AAT  
816

55

Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly Glu Ser Asn  
 260 265 270  
 CTA AAT GTT TTA TAT AAT TGC ACT GTG GCC AGC ACG GGA GGC ACA GAC  
 5 864  
 Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly Gly Thr Asp  
 275 280 285  
 ACC AAA AGC TTC ATC TTG GTG AGA AAA GAC ATG GCT GAT ATC CCA GGC  
 912  
 Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp Ile Pro Gly  
 10 290 295 300  
 CAC GTC TTC ACA AGA  
 927  
 His Val Phe Thr Arg  
 305

15 (8) INFORMATION FOR SEQ ID NO:7:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:1620 base pairs  
 (B) TYPE:nucleic acid  
 (C) strandedness:double  
 (D) TOPOLOGY:linear  
 20 (ii) MOLECULE TYPE:cDNA  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM:lymphoblastoid cell derived from a patient  
 with Hodgkin's disease  
 (B) INDIVIDUAL ISOLATE:L428 (FERM BP-5777)  
 25 (ix) FEATURE:  
 (A) NAME/KEY:sig peptide  
 (B) LOCATION:1..57  
 (C) IDENTIFICATION METHOD:E  
 (ix) FEATURE:  
 (A) NAME/KEY:mat peptide  
 (B) LOCATION:58..1620  
 30 (C) IDENTIFICATION METHOD:E  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA  
 48  
 Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val  
 35 -15 -10 -5  
 AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA  
 96  
 Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu  
 1 5 10  
 GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG  
 40 144  
 Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu  
 15 20 25  
 ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA  
 192  
 Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu  
 45 30 35 40 45  
 CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT  
 240  
 His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp  
 50 55 60  
 TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC  
 50 288  
 Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr  
 65 70 75  
 TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC

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EP 0 864 585 A1

336  
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile  
80 85 90  
5 AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA  
384  
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys  
95 100 105  
ATT GTG GAA GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC  
432  
10 Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr  
110 115 120 125  
TAT CAA ACA CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG  
480  
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys  
130 135 140  
15 CTA CTA CTG GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG  
528  
Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu  
145 150 155  
TTT GAA GAT CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT  
576  
20 Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn  
160 165 170  
GGA AAA CTA TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA  
624  
Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu  
175 180 185  
25 GAT CGC AGT AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT  
672  
Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His  
190 195 200 205  
GTT GCA GTG GAA TTA GGA AAA AAC GTA AGG CTC AAC TGC TCT GCT TTG  
720  
30 Val Ala Val Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu  
210 215 220  
CTG AAT GAA GAG GAT GTA ATT TAT TGG ATG TTC GGG GAA GAA AAT GGA  
768  
Leu Asn Glu Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly  
225 230 235  
35 TCG GAT CCT AAT ATA CAT GAA GAG AAA GAA ATG AGA ATT ATG ACT CCA  
816  
Ser Asp Pro Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro  
240 245 250  
GAA GGC AAA TGG CAT GCT TCA AAA GTA TTG AGA ATT GAA AAT ATT GGT  
864  
40 Glu Gly Lys Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly  
255 260 265  
GAA AGC AAT CTA AAT GTT TTA TAT AAT TGC ACT GTG GCC AGC ACG GGA  
912  
Glu Ser Asn Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly  
270 275 280 285  
45 GGC ACA GAC ACC AAA AGC TTC ATC TTG GTG AGA AAA GAC ATG GCT GAT  
960  
Gly Thr Asp Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp  
290 295 300  
ATC CCA GGC CAC GTC TTC ACA AGA GGA ATG ATC ATA GCT GTT TTG ATC  
1008  
50 Ile Pro Gly His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu Ile  
305 310 315  
TTG GTG GCA GTA GTG TGC CTA GTG ACT GTG TGT GTC ATT TAT AGA GTT  
1056

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# EP 0 864 585 A1

5 Leu Val Ala Val Val Cys Leu Val Thr Val Cys Val Ile Tyr Arg Val  
 320 325 330  
 GAC TTG GTT CTA TTT TAT AGA CAT TTA ACG AGA AGA GAT GAA ACA TTA  
 1104  
 Asp Leu Val Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr Leu  
 335 340 345  
 ACA GAT GGA AAA ACA TAT GAT GCT TTT GTG TCT TAC CTA AAA GAA TGC  
 1152  
 10 Thr Asp Gly Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys  
 350 355 360  
 CGA CCT GAA AAT GGA GAG GAG CAC ACC TTT GCT GTG GAG ATT TTG CCC  
 1200  
 Arg Pro Glu Asn Gly Glu Glu His Thr Phe Ala Val Glu Ile Leu Pro  
 370 375 380  
 AGG GTG TTG GAG AAA CAT TTT GGG TAT AAG TTA TGC ATA TTT GAA AGG  
 1248  
 15 Arg Val Leu Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg  
 385 390 395  
 GAT GTA GTG CCT GGA GGA GCT GTT GTT GAT GAA ATC CAC TCA CTG ATA  
 1296  
 20 Asp Val Val Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu Ile  
 400 405 410  
 GAG AAA AGC CGA AGA CTA ATC ATT GTC CTA AGT AAA AGT TAT ATG TCT  
 1344  
 Glu Lys Ser Arg Arg Leu Ile Ile Val Leu Ser Lys Ser Tyr Met Ser  
 415 420 425  
 AAT GAG GTC AGG TAT GAA CTT GAA AGT GGA CTC CAT GAA GCA TTG GTG  
 1392  
 25 Asn Glu Val Arg Tyr Glu Leu Glu Ser Gly Leu His Glu Ala Leu Val  
 430 435 440  
 GAA AGA AAA ATT AAA ATA ATC TTA ATT GAA TTT ACA CCT GTT ACT GAC  
 1440  
 Glu Arg Lys Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr Asp  
 450 455 460  
 30 TTC ACA TTC TTG CCC CAA TCA CTA AAG CTT TTG AAA TCT CAC AGA GTT  
 1488  
 Phe Thr Phe Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg Val  
 465 470 475  
 CTG AAG TGG AAG GCC GAT AAA TCT CTT TCT TAT AAC TCA AGG TTC TGG  
 1536  
 35 Leu Lys Trp Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe Trp  
 480 485 490  
 AAG AAC CTT CTT TAC TTA ATG CCT GCA AAA ACA GTC AAG CCA GGT AGA  
 1584  
 40 Lys Asn Leu Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly Arg  
 495 500 505  
 GAC GAA CCG GAA GTC TTG CCT GTT CTT TCC GAG TCT  
 1620  
 Asp Glu Pro Glu Val Leu Pro Val Leu Ser Glu Ser  
 510 515 520

45 (9) INFORMATION FOR SEQ ID NO:8:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:369 base pairs  
 (B) TYPE:nucleic acid  
 (C) strandedness:double  
 50 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:cdna  
 (ix) FEATURE:  
 (A) NAME/KEY:sig peptide

55

(B) LOCATION:1..57  
 (C) IDENTIFICATION METHOD:S  
 (ix) FEATURE:  
 (A) NAME/KEY:mat peptide  
 (B) LOCATION:58..369  
 (C) IDENTIFICATION METHOD:S  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

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ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA
48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val
-15 -10 -5
AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA
96
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu
1 5 10
GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG
144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu
15 20 25
ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA
192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu
30 35 40 45
CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT
240
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp
50 55 60
TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC
288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr
65 70 75
TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC
336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile
80 85 90
AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA
369
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg
95 100

```

(10) INFORMATION FOR SEQ ID NO:9:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:678 base pairs  
 (B) TYPE:nucleic acid  
 (C) strandedness:double  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:cDNA  
 (ix) FEATURE:  
 (A) NAME/KEY:sig peptide  
 (B) LOCATION:1..57  
 (C) IDENTIFICATION METHOD:S

(ix) FEATURE:  
 (A) NAME/KEY:mat peptide  
 (B) LOCATION:58..678  
 (C) IDENTIFICATION METHOD:S  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

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ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA
48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val

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EP 0 864 585 A1

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 -15 -10 -5
AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA
96
5 Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu
 1 5 10
GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG
144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu
 15 20 25
10 ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA
192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu
 30 35 40 45
CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT
240
15 His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp
 50 55 60
TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC
288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr
 65 70 75
20 TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC
336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile
 80 85 90
AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA
384
25 Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys
 95 100 105
ATT GTG GAA GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC
432
Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr
 110 115 120 125
30 TAT CAA ACA CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG
480
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys
 130 135 140
CTA CTA CTG GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG
528
35 Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu
 145 150 155
TTT GAA GAT CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT
576
Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn
 160 165 170
40 GGA AAA CTA TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA
624
Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu
 175 180 185
GAT CGC AGT AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT
672
45 Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His
 190 195 200 205
GTT GCA
678
Val Ala

```

(11) INFORMATION FOR SEQ ID NO:10:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:984 base pairs  
 (B) TYPE:nucleic acid

(C) strandedness:double  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:cDNA  
 (ix) FEATURE:  
 (A) NAME/KEY:sig peptide  
 (B) LOCATION:1..57  
 (C) IDENTIFICATION METHOD:E  
 (ix) FEATURE:  
 (A) NAME/KEY:mat peptide  
 (B) LOCATION:58..984  
 (C) IDENTIFICATION METHOD:E  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

```

ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA
48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val
15
AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA
96
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu
20
GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG
144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu
15 20 25
ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA
192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu
25
30 35 40 45
CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT
240
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp
50 55 60
TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC
30
288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr
65 70 75
TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC
336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile
80 85 90
AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA
384
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys
95 100 105
ATT GTG GAA GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC
40
432
Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr
110 115 120 125
TAT CAA ACA CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG
480
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys
130 135 140
CTA CTA CTG GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG
528
Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu
145 150 155
50
TTT GAA GAT CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT AAT
576
Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn
160 165 170

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5 GGA AAA CTA TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA  
 624  
 Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu  
 175 180 185  
 GAT CGC AGT AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT  
 672  
 Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His  
 190 195 200 205  
 GTT GCA GTG GAA TTA GGA AAA AAC GTA AGG CTC AAC TGC TCT GCT TTG  
 720  
 10 Val Ala Val Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu  
 210 215 220  
 CTG AAT GAA GAG GAT GTA ATT TAT TGG ATG TTC GGG GAA GAA AAT GGA  
 768  
 Leu Asn Glu Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly  
 225 230 235  
 15 TCG GAT CCT AAT ATA CAT GAA GAG AAA GAA ATG AGA ATT ATG ACT CCA  
 816  
 Ser Asp Pro Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro  
 240 245 250  
 GAA GGC AAA TGG CAT GCT TCA AAA GTA TTG AGA ATT GAA AAT ATT GGT  
 864  
 20 Glu Gly Lys Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly  
 255 260 265  
 GAA AGC AAT CTA AAT GTT TTA TAT AAT TGC ACT GTG GCC AGC ACG GGA  
 912  
 Glu Ser Asn Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly  
 270 275 280 285  
 25 GGC ACA GAC ACC AAA AGC TTC ATC TTG GTG AGA AAA GAC ATG GCT GAT  
 960  
 Gly Thr Asp Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp  
 290 295 300  
 ATC CCA GGC CAC GTC TTC ACA AGA  
 984  
 30 Ile Pro Gly His Val Phe Thr Arg  
 305

(12) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 975 base pairs

(B) TYPE: nucleic acid

(C) strandedness: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: sig peptide

(B) LOCATION: 1..54

(C) IDENTIFICATION METHOD: S

(ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 55..975

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

48  
 ATG CAT CAT GAA GAA TTA ATC TTG ACA CTC TGC ATT CTC ATT GTT AAA  
 50 Met His His Glu Glu Leu Ile Leu Thr Leu Cys Ile Leu Ile Val Lys  
 -15 -10 -5  
 AGT GCC TCA AAA AGT TGT ATT CAC CGA TCA CAA ATT CAT GTG GTA GAG  
 96  
 Ser Ala Ser Lys Ser Cys Ile His Arg Ser Gln Ile His Val Val Glu

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EP 0 864 585 A1

1 5 10  
 GGA GAA CCT TTT TAT CTG AAG CCA TGT GGC ATA TCT GCA CCA GTG CAC  
 144  
 5 Gly Glu Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His  
 15 20 25 30  
 AGG AAT GAA ACA GCC ACC ATG AGA TGG TTC AAA GGC AGT GCT TCA CAT  
 192  
 Arg Asn Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His  
 35 40 45  
 10 GAG TAT AGA GAG CTG AAC AAC AGA AGC TCG CCC AGA GTC ACT TTT CAT  
 240  
 Glu Tyr Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His  
 50 55 60  
 GAT CAC ACC TTG GAA TTC TGG CCA GTT GAG ATG GAG GAT GAG GGA ACG  
 288  
 15 Asp His Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr  
 65 70 75  
 TAC ATT TCT CAA GTC GGA AAT GAT CGT CGC AAT TGG ACC TTA AAT GTC  
 336  
 Tyr Ile Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val  
 80 85 90  
 20 ACC AAA AGA AAC AAA CAC AGC TGT TTC TCT GAC AAG CTC GTG ACA AGC  
 384  
 Thr Lys Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser  
 95 100 105 110  
 AGA GAT GTT GAA GTT AAC AAA TCT CTG CAT ATC ACT TGT AAG AAT CCT  
 432  
 25 Arg Asp Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro  
 115 120 125  
 AAC TAT GAA GAG CTG ATC CAG GAC ACA TGG CTG TAT AAG AAC TGT AAG  
 480  
 Asn Tyr Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys  
 130 135 140  
 30 GAA ATA TCC AAA ACC CCA AGG ATC CTG AAG GAT GCC GAG TTT GGA GAT  
 528  
 Glu Ile Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp  
 145 150 155  
 GAG GGC TAC TAC TCC TGC GTG TTT TCT GTC CAC CAT AAT GGG ACA CGG  
 576  
 35 Glu Gly Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg  
 160 165 170  
 TAC AAC ATC ACC AAG ACT GTC AAT ATA ACA GTT ATT GAA GGA AGG AGT  
 624  
 Tyr Asn Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser  
 175 180 185 190  
 40 AAA GTA ACT CCA GCT ATT TTA GGA CCA AAG TGT GAG AAG GTT GGT GTA  
 672  
 Lys Val Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val  
 195 200 205  
 GAA CTA GGA AAG GAT GTG GAG TTG AAC TGC AGT GCT TCA TTG AAT AAA  
 720  
 45 Glu Leu Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys  
 210 215 220  
 GAC GAT CTG TTT TAT TGG AGC ATC AGG AAA GAG GAC AGC TCA GAC CCT  
 768  
 Asp Asp Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro  
 225 230 235  
 50 AAT GTG CAA GAA GAC AGG AAG GAG ACG ACA ACA TGG ATT TCT GAA GGC  
 816  
 Asn Val Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly  
 240 245 250

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AAA CTG CAT GCT TCA AAA ATA CTG AGA TTT CAG AAA ATT ACT GAA AAC  
 864  
 Lys Leu His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn  
 5 255 260 265 270  
 TAT CTC AAT GTT TTA TAT AAT TGC ACC GTG GCC AAC GAA GAA GCC ATA  
 912  
 Tyr Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile  
 275 280 285  
 GAC ACC AAG AGC TTC GTC TTG GTG AGA AAA GAA ATA CCT GAT ATC CCA  
 10 960  
 Asp Thr Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro  
 290 295 300  
 GGC CAT GTC TTT ACA  
 975  
 Gly His Val Phe Thr  
 15 305

(13) INFORMATION FOR SEQ ID NO:12:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:5  
 (B) TYPE:amino acid  
 20 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

Trp His Ala Ser Lys  
 25 1

(14) INFORMATION FOR SEQ ID NO:13:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:7  
 (B) TYPE:amino acid  
 30 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

Ile Met Thr Pro Glu Gly Lys  
 35 1 5

(15) INFORMATION FOR SEQ ID NO:14:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:13  
 (B) TYPE:amino acid  
 40 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

Ser Ser Gly Ser Gln Glu His Val Glu Leu Asn Pro Arg  
 45 1 5 10

(16) INFORMATION FOR SEQ ID NO:15:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:4  
 (B) TYPE:amino acid  
 50 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

55

Ser Trp Tyr Lys  
1

5 (17) INFORMATION FOR SEQ ID NO:16:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:10  
 (B) TYPE:amino acid  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 10 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:16:

Leu Asn His Val Ala Val Glu Leu Gly Lys  
1 5 10

15 (18) INFORMATION FOR SEQ ID NO:17:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:6  
 (B) TYPE:amino acid  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 20 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:17:

Ser Phe Ile Leu Val Arg  
1 5

25 (19) INFORMATION FOR SEQ ID NO:18:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:15  
 (B) TYPE:amino acid  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 30 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:18:

Thr Val Lys Pro Gly Arg Asp Glu Pro Glu Val Leu Pro Val Leu  
1 5 10 15

35 (20) INFORMATION FOR SEQ ID NO:19:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:11  
 (B) TYPE:amino acid  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 40 (v) FRAGMENT TYPE:internal fragment  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:19:

Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys  
1 5 10

45 (21) INFORMATION FOR SEQ ID NO:20:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:521  
 (B) TYPE:amino acid  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 50 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro  
1 5 10 15

55

EP 0 864 585 A1

Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr  
 Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu  
 5 Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu  
 Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln  
 Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn  
 10 Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu  
 Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr  
 Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu  
 15 Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp  
 Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu  
 Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser  
 20 Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala Val  
 Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu Leu Asn Glu  
 Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly Ser Asp Pro  
 25 Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro Glu Gly Lys  
 Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly Glu Ser Asn  
 Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly Gly Thr Asp  
 30 Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp Ile Pro Gly  
 His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu Ile Leu Val Ala  
 Val Val Cys Leu Val Thr Val Cys Val Ile Tyr Arg Val Asp Leu Val  
 35 Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr Leu Thr Asp Gly  
 Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys Arg Pro Glu  
 Asn Gly Glu Glu His Thr Phe Ala Val Glu Ile Leu Pro Arg Val Leu  
 40 Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg Asp Val Val  
 Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu Ile Glu Lys Ser  
 Arg Arg Leu Ile Ile Val Leu Ser Lys Ser Tyr Met Ser Asn Glu Val  
 45 Arg Tyr Glu Leu Glu Ser Gly Leu His Glu Ala Leu Val Glu Arg Lys  
 Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr Asp Phe Thr Phe  
 Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg Val Leu Lys Trp  
 50 Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe Trp Lys Asn Leu  
 Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly Arg Asp Glu Pro

55

500 505 510  
 Glu Val Leu Pro Val Leu Ser Glu Ser  
 515 520

(22) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:519

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

Ser Lys Ser Cys Ile His Arg Ser Gln Ile His Val Val Glu Gly Glu  
 1 5 10 15  
 Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His Arg Asn  
 20 25 30  
 Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr  
 35 40 45  
 Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His Asp His  
 50 55 60  
 Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile  
 65 70 75 80  
 Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val Thr Lys  
 85 90 95  
 Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser Arg Asp  
 100 105 110  
 Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro Asn Tyr  
 115 120 125  
 Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys Glu Ile  
 130 135 140  
 Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly  
 145 150 155 160  
 Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg Tyr Asn  
 165 170 175  
 Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser Lys Val  
 180 185 190  
 Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val Glu Leu  
 195 200 205  
 Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys Asp Asp  
 210 215 220  
 Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro Asn Val  
 225 230 235 240  
 Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly Lys Leu  
 245 250 255  
 His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn Tyr Leu  
 260 265 270  
 Asn Val leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile Asp Thr  
 275 280 285  
 Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His  
 290 295 300  
 Val Phe Thr Gly Gly Val Thr Val Leu Val Leu Ala Ser Val Ala Ala  
 305 310 315 320  
 Val Cys Ile Val Ile Leu Cys Val Ile Tyr Lys Val Asp Leu Val Leu  
 325 330 335  
 Phe Tyr Arg Arg Ile Ala Glu Arg Asp Glu Thr Leu Thr Asp Gly Lys  
 340 345 350  
 Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys His Pro Glu Asn  
 355 360 365  
 Lys Glu Glu Tyr Thr Phe Ala Val Glu Thr Leu Pro Arg Val Leu Glu  
 370 375 380  
 Lys Gln Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg Asp Val Val Pro

EP 0 864 585 A1

385 390 395 400  
 Gly Gly Ala Val Val Glu Glu Ile His Ser Leu Ile Glu Lys Ser Arg  
 405 410 415  
 5 Arg Leu Ile Ile Val Leu Ser Gln Ser Tyr Leu Thr Asn Gly Ala Arg  
 420 425 430  
 Arg Glu Leu Glu Ser Gly Leu His Glu Ala Leu Val Glu Arg Lys Ile  
 435 440 445  
 Lys Ile Ile Leu Ile Glu Phe Thr Pro Ala Ser Asn Ile Thr Phe Leu  
 450 455 460  
 10 Pro Pro Ser Leu Lys Leu Lys Ser Tyr Arg Val Leu Lys Trp Arg  
 465 470 475 480  
 Ala Asp Ser Pro Ser Met Asn Ser Arg Phe Trp Lys Asn Leu Val Tyr  
 485 490 495  
 Leu Met Pro Ala Lys Ala Val Lys Pro Trp Arg Glu Glu Ser Glu Ala  
 500 505 510  
 15 Arg Ser Val Leu Ser Ala Pro  
 515

(23) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:309

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:22:

25 Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro  
 1 5 10 15  
 Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr  
 20 25 30  
 Thr Thr Lys Ser Trp Tyr Lys Ser Gly Ser Gln Glu His Val Glu  
 35 40 45  
 Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu  
 50 55 60  
 30 Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln  
 65 70 75 80  
 Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn  
 85 90 95  
 Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu  
 100 105 110  
 35 Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr  
 115 120 125  
 Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu  
 130 135 140  
 40 Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp  
 145 150 155 160  
 Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu  
 165 170 175  
 Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser  
 180 185 190  
 Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala Val  
 195 200 205  
 45 Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu Leu Asn Glu  
 210 215 220  
 Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly Ser Asp Pro  
 225 230 235 240  
 Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro Glu Gly Lys  
 245 250 255  
 50 Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly Glu Ser Asn  
 260 265 270  
 Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly Gly Thr Asp

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275 280 285  
 Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp Ile Pro Gly  
 290 295 300  
 5 His Val Phe Thr Arg  
 305

## (24) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:207

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

15 Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro  
 1 5 10 15  
 Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr  
 20 20 25 30  
 Thr Thr Lys Ser Trp Tyr Lys Ser Gly Ser Gln Glu His Val Glu  
 35 40 45  
 Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu  
 50 55 60  
 20 Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln  
 65 70 75 80  
 Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn  
 85 90 95  
 Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu  
 100 105 110  
 25 Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr  
 115 120 125  
 Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu  
 130 135 140  
 30 Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp  
 145 150 155 160  
 Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu  
 165 170 175  
 Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser  
 180 185 190  
 Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala  
 195 200 205  
 35

## (25) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:104

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

45 Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro  
 1 5 10 15  
 Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr  
 20 25 30  
 The Thr Lys Ser Trp Tyr Lys Ser Gly Ser Gln Glu His Val Glu  
 35 40 45  
 Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu  
 50 55 60  
 50 Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln  
 65 70 75 80  
 Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn  
 85 90 95



Lys His Ser Cys Phe Thr Glu Arg  
100

- 5 (26) INFORMATION FOR SEQ ID NO:25:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:307  
 (B) TYPE:amino acid  
 (D) TOPOLOGY:linear  
 (ii) MOLECULE TYPE:peptide  
 10 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:25:

Ser Lys Ser Cys Ile His Arg Ser Gln Ile His Val Val Glu Gly Glu  
1 5 10 15  
 Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His Arg Asn  
20 25 30  
 15 Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr  
35 40 45  
 Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His Asp His  
50 55 60  
 Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile  
65 70 75 80  
 20 Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val Thr Lys  
85 90 95  
 Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser Arg Asp  
100 105 110  
 Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro Asn Tyr  
115 120 125  
 25 Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys Glu Ile  
130 135 140  
 Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly  
145 150 155 160  
 Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg Tyr Asn  
165 170 175  
 30 Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser Lys Val  
180 185 190  
 Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val Glu Leu  
195 200 205  
 Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys Asp Asp  
210 215 220  
 35 Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro Asn Val  
225 230 235 240  
 Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly Lys Leu  
245 250 255  
 His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn Tyr Leu  
260 265 270  
 40 Asn Val Leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile Asp Thr  
275 280 285  
 Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His  
290 295 300  
 Val Phe Thr  
305

- 45 (27) INFORMATION FOR SEQ ID NO:26:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH:157  
 (B) TYPE:amino acid  
 (D) TOPOLOGY:linear  
 50 (ii) MOLECULE TYPE:peptide  
 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:26:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn

55

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 1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
 20 25 30
5 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
 35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
 50 55 60
Ser Val Lys Cys Glu Lys Ile Ser Xaa Leu Ser Cys Glu Asn Lys Ile
 65 70 75 80
10 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
 85 90 95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
 100 105 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
 115 120 125
15 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
 130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
 145 150 155

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20 (28) INFORMATION FOR SEQ ID NO:27:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH:157
 (B) TYPE:amino acid
 (D) TOPOLOGY:linear
 (ii) MOLECULE TYPE:peptide
25 (xi) SEQUENCE DESCRIPTION:SEQ ID NO:27:

```

```

Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn
 1 5 10 15
Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
 20 25 30
30 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile
 35 40 45
Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser
 50 55 60
Val Lys Asp Ser Lys Xaa Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile
 65 70 75 80
35 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser
 85 90 95
Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu
 100 105 110
Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu
 115 120 125
40 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp
 130 135 140
Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser
 145 150 155

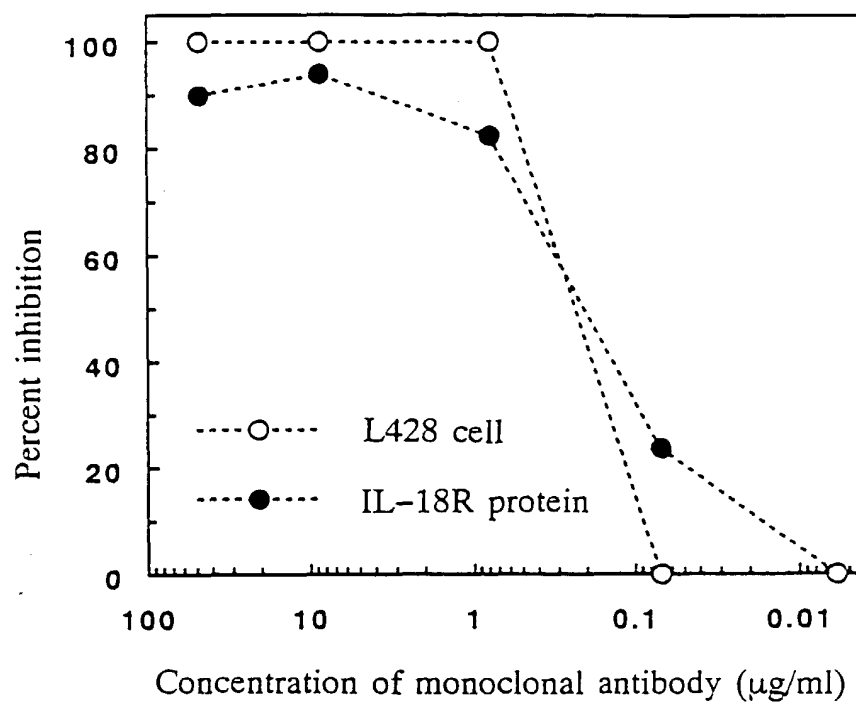
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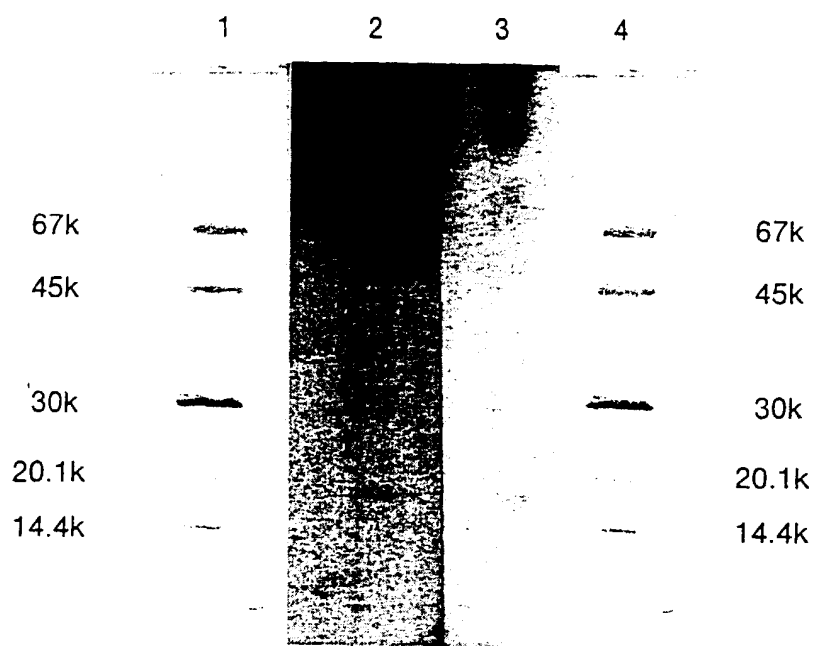
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## Claims

- 50 1. A polypeptide as interleukin-18 receptor, which is obtainable through gene expression.
2. The polypeptide of claim 1, which is obtainable by bringing into expression a human or mouse gene in a host of animal, plant or microbe origin.
- 55 3. The polypeptide of claim 1, wherein said gene contains a part or whole of the nucleotide sequence of SEQ ID NO:1 or 2.
4. The polypeptide of claim 1, wherein said gene contains the nucleotide sequence of SEQ ID NO:3 or 4.

5. The polypeptide of claim 1, wherein said gene contains either nucleotide sequence of SEQ ID NOs:5 to 11.
6. The polypeptide of claim 1, which contains one or more amino acid sequences of SEQ ID NOs:12 to 19.
- 5 7. The polypeptide of claim 1, which contains a part or whole of the nucleotide sequence of SEQ ID NO:20 or 21.
8. The polypeptide of claim 1, which contains either amino acid sequence of SEQ ID NOs:22 to 25.
9. An agent for interleukin-18 receptor susceptible diseases, which contains as effective ingredient the polypeptide of  
10 claim 1.
10. The agent of claim 9, which contains as stabilizer a protein, saccharide and/or buffer.
11. An anti-autoimmune disease agent in accordance with claim 9.
- 15 12. An immunosuppressant in accordance with claim 9.
13. A DNA which encodes the polypeptide of claim 1.
- 20 14. The DNA of claim 13, which originates from human or mouse.
15. The DNA of claim 13, which contains a part or whole of either nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2  
or their complementary sequence.
- 25 16. The DNA of claim 13, which contains either nucleotide sequence of SEQ ID NO:3, SEQ ID NO:4 or their comple-  
mentary sequence.
17. The DNA of claim 13, which contains either nucleotide sequence of SEQ ID NO: 5, SEQ ID: 6, SEQ ID NO: 7, SEQ  
ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11 or their complementary sequence.
- 30 18. The DNA of claim 13, wherein, based on the degeneracy of genetic codes, one or more nucleotides are replaced  
with different nucleotides while conserving the amino acid sequence.
19. The DNA of claim 13, which is placed in an autonomously replicable vector.
- 35 20. The DNA of claim 13, which is placed in a host of animal, plant or microbe origin.
21. A process to prepare polypeptide, comprising bringing into expression a DNA which encodes the polypeptide of  
claim 1; and collecting the resultant polypeptide.
- 40 22. The process of claim 21, wherein the resultant polypeptide is collected through a step which includes salting out,  
dialysis, filtration, concentration, fractional precipitation, ion-exchange chromatography, gel filtration chromatogra-  
phy, adsorption chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed  
phase chromatography, affinity chromatography, gel electrophoresis and/or isoelectric focusing gel electrophoresis.
- 45 23. The process of claim 21, wherein the resultant polypeptide is collected through a step which includes immunoaffin-  
ity chromatography using monoclonal antibody.
24. A agent to neutralize interleukin-18, which contains as effective ingredient the polypeptide of claim 1.
- 50 25. A method to neutralize interleukin-18, characterized by allowing the polypeptide of claim 1 to act on interleukin-18.

FIG. 1



Lane 1 : Molecular weight markers  
 Lane 2 : Sample (with monoclonal antibody)  
 Lane 3 : Sample (without monoclonal antibody)  
 Lane 4 : Molecular weight markers

FIG. 2

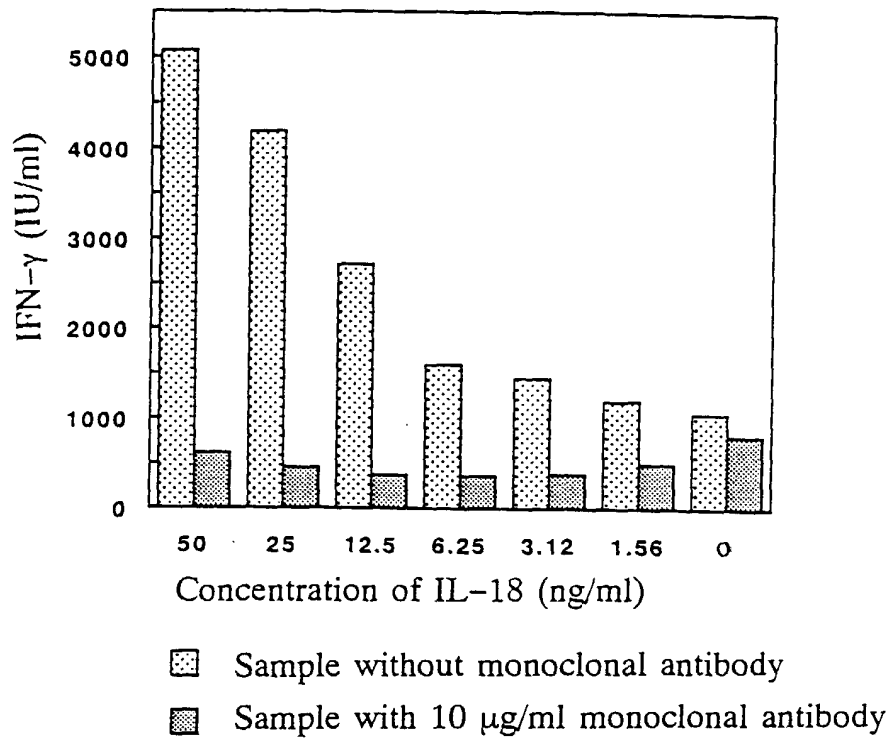


FIG. 3

Absorbance at 280 nm ..... IL-18R protein content (cpm)....o...

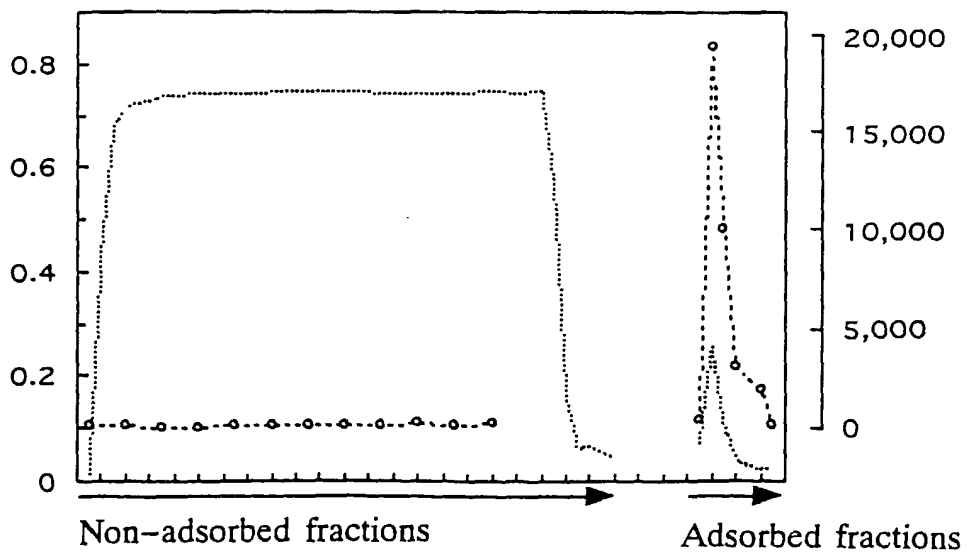
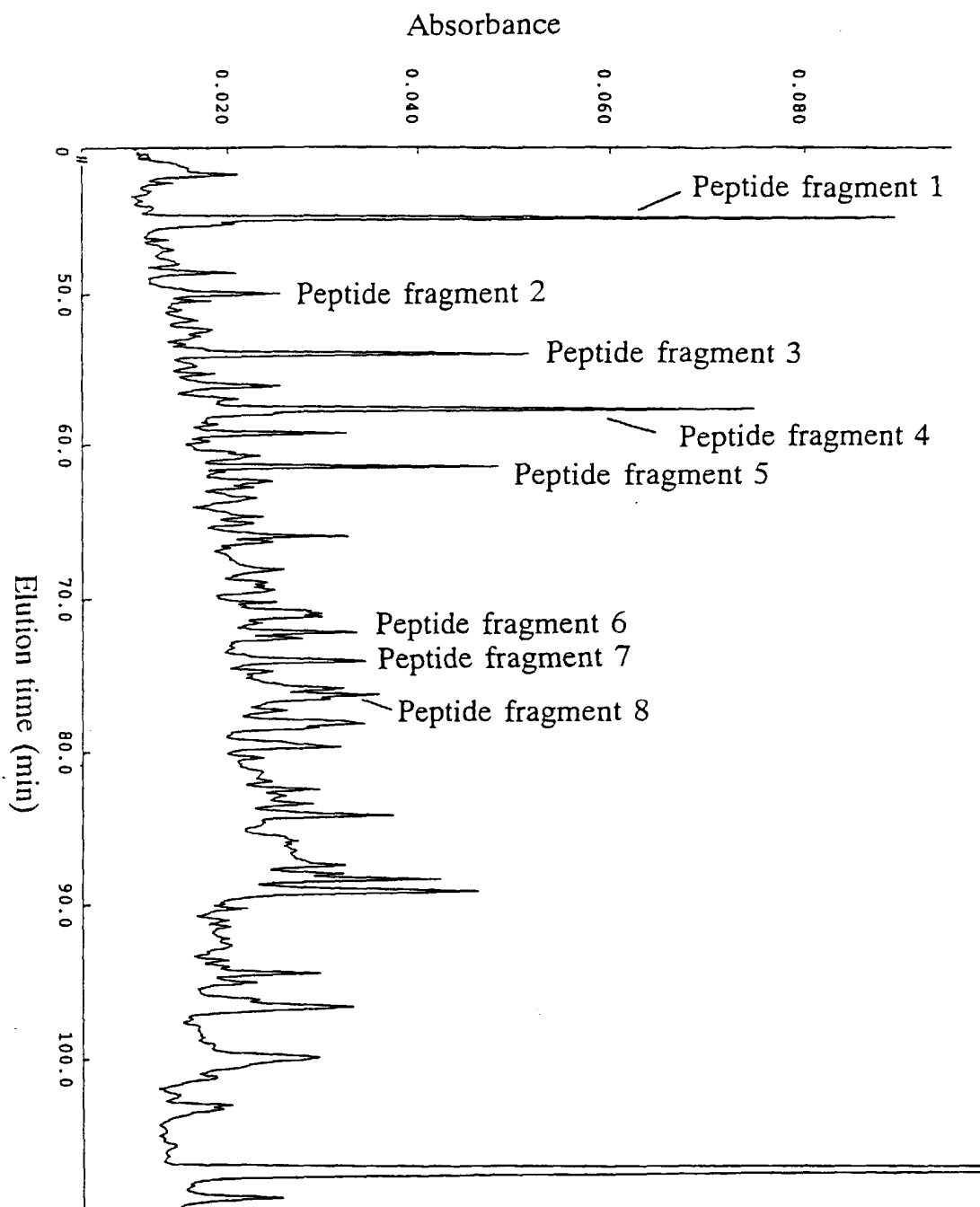
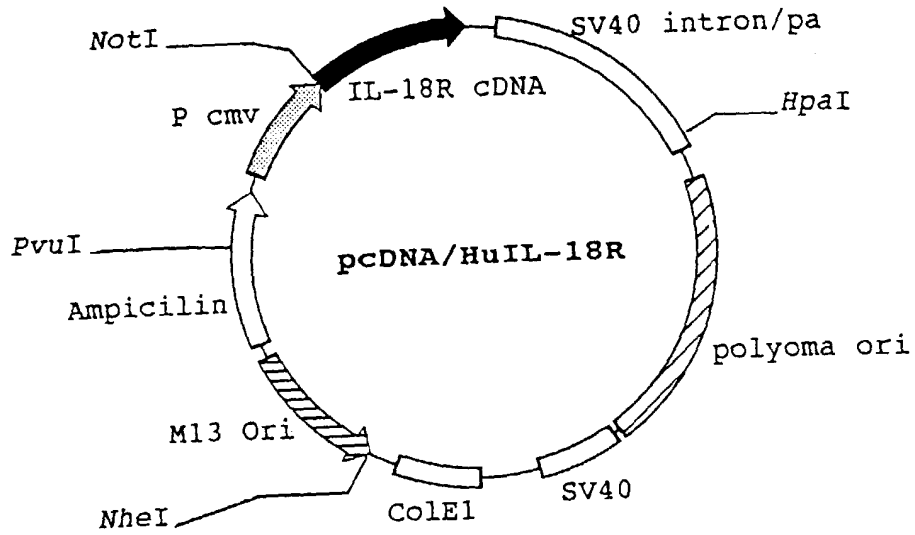
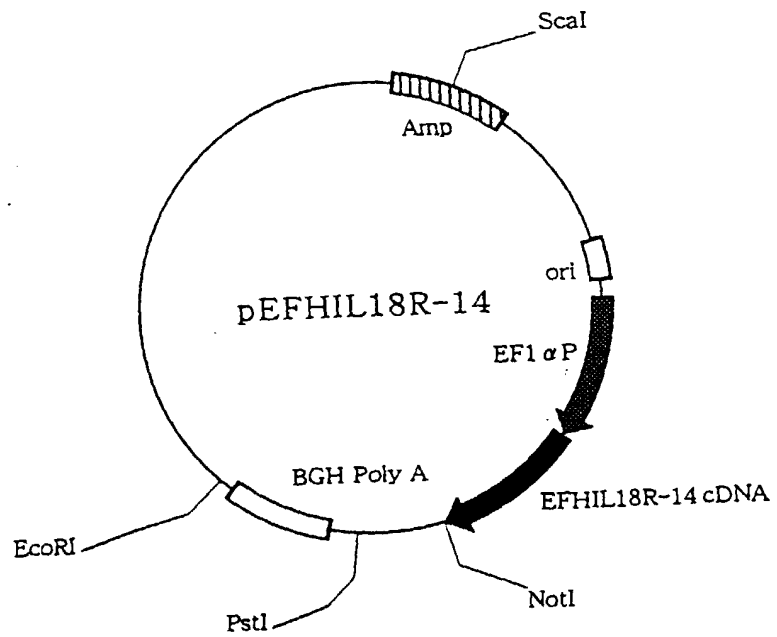


FIG. 4

FIG. 5



**FIG. 6**



**FIG. 7**



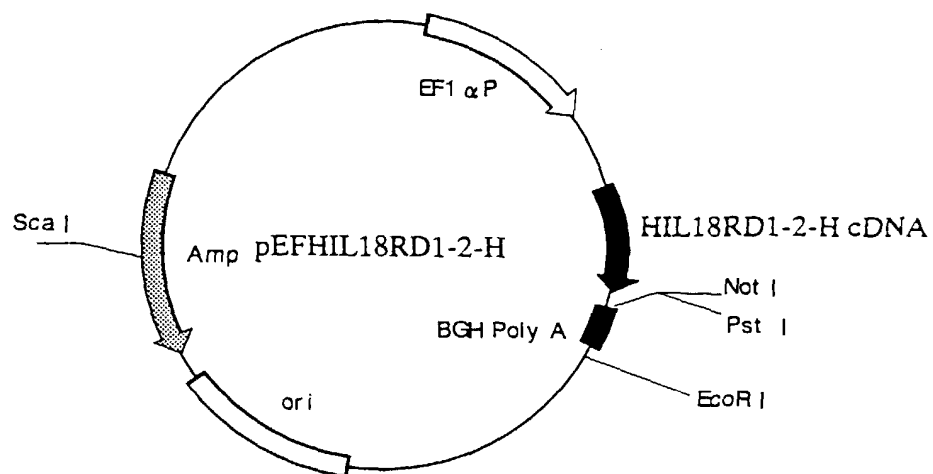


FIG. 8

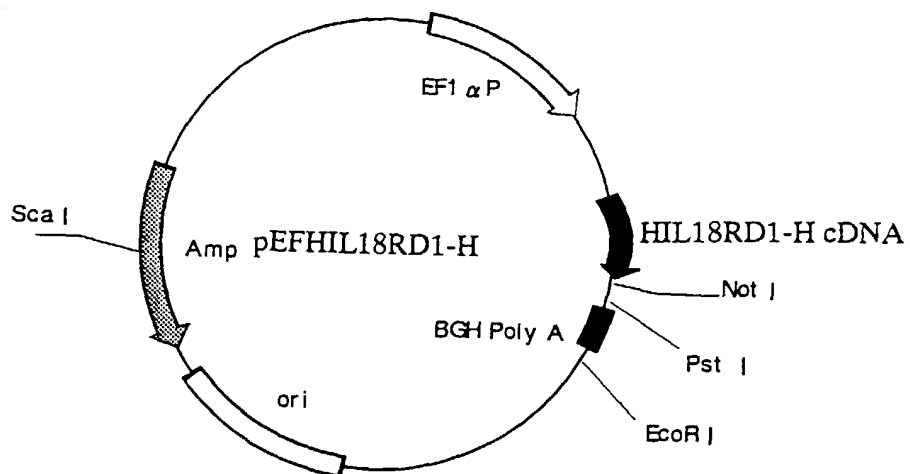


FIG. 9

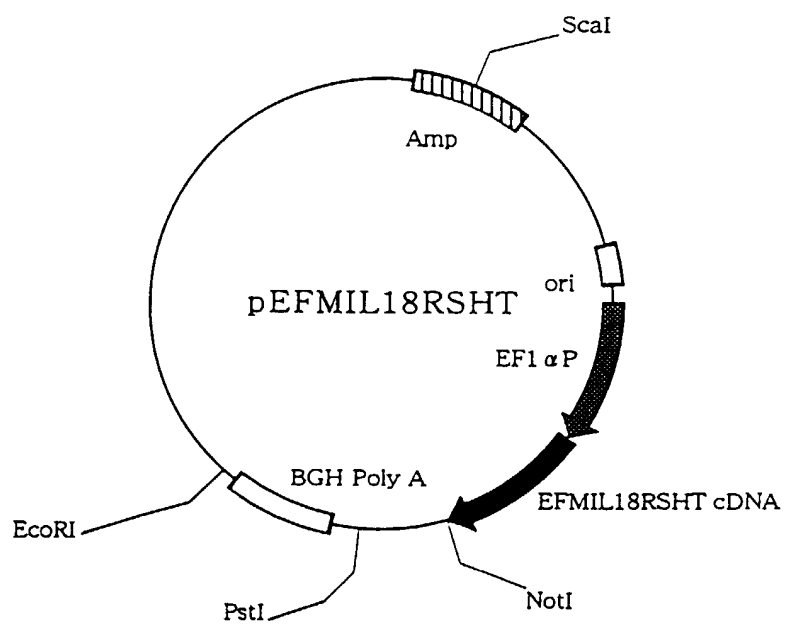


FIG. 10



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 97 31 0517

| DOCUMENTS CONSIDERED TO BE RELEVANT                                                                                                                                                                                     |                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                              |                                              |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
| Category                                                                                                                                                                                                                | Citation of document with indication, where appropriate, of relevant passages                                                                                                                                                          | Relevant to claim                                                                                                                                                                                                                                                            | CLASSIFICATION OF THE APPLICATION (Int.Cl.6) |
| X                                                                                                                                                                                                                       | PARNET, P. ET AL.: "IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologs T1/ST2 and IL-1R AcP"<br>J. BIOL. CHEM., 271(8), 3967-70,<br>23 February 1996, XP002059933<br>* figure 1 * | 1-11,27                                                                                                                                                                                                                                                                      | C07K14/715<br>A61K38/17<br>A61K39/00         |
| P,X                                                                                                                                                                                                                     | WO 97 31010 A (IMMUNEX CORP) 28 August 1997<br>* the whole document *                                                                                                                                                                  | 1-28                                                                                                                                                                                                                                                                         |                                              |
|                                                                                                                                                                                                                         |                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                              | TECHNICAL FIELDS SEARCHED (Int.Cl.6)         |
|                                                                                                                                                                                                                         |                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                              | C07K<br>A61K                                 |
| The present search report has been drawn up for all claims                                                                                                                                                              |                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                              |                                              |
| Place of search<br>MUNICH                                                                                                                                                                                               |                                                                                                                                                                                                                                        | Date of completion of the search<br>25 March 1998                                                                                                                                                                                                                            | Examiner<br>Chakravarty, A                   |
| CATEGORY OF CITED DOCUMENTS                                                                                                                                                                                             |                                                                                                                                                                                                                                        | T : theory or principle underlying the invention<br>E : earlier patent document, but published on, or after the filing date<br>D : document cited in the application<br>L : document cited for other reasons<br>& : member of the same patent family, corresponding document |                                              |
| X : particularly relevant if taken alone<br>Y : particularly relevant if combined with another document of the same category<br>A : technological background<br>O : non-written disclosure<br>P : intermediate document |                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                              |                                              |

EPO FORM 1503 03.82 (P04C01)